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OVERWINTERING OF THE HOUSE FLY

By R. H. HUTCHISON,[1] *Scientific Assistant, Bureau of Entomology, United States Department of Agriculture*

INTRODUCTION

Experiments and observations bearing on the problem of the method of the overwintering of the house fly (*Musca domestica* Linnaeus) were begun by the Bureau of Entomology during the fall of 1914 at the Arlington Experimental Farm of the Bureau of Plant Industry. The following spring the work was transferred to the experiment station of the Bureau of Animal Industry at Bethesda, Md., and was continued there during the two seasons 1915–17.

OVERWINTERING OF ADULT FLIES UNDER EXPERIMENTAL CONDITIONS

UNDER OUTDOOR CONDITIONS

A large number of experiments were conducted during the winter of 1914–15. Reared flies were put in screen-wire cages, which measured 10 by 12 by 22 inches. In the bottom of each was a drawer in which food and water were supplied. The food materials usually consisted of sliced banana and fresh horse manure. The cages were kept on shelves of a screened insectary where they were subject to outdoor temperature and humidity, but were protected from rain and to some extent from direct sunlight. The banana and manure were renewed at frequent intervals so that a fresh food supply was always available. The most significant results of these experiments are summarized in Table I.

[1] The writer wishes to express his appreciation of the kindness of Mr. E. C. Butterfield, superintendent of the Arlington Experimental Farm near Rosslyn, Va., and Dr. E. C. Schroeder, superintendent of the experiment station of the Bureau of Animal Industry at Bethesda, Md., for the facilities afforded during the course of the work. The writer's thanks are also due to Dr. C. H. T. Townsend, of the Bureau of Entomology, for determining many specimens of Muscidae, and to Mr. Frederick Knab, of the Bureau of Entomology, for the determination of many nonmuscids.

TABLE I.—*Longevity of house flies during winter in cages under outdoor conditions*

Experiment No.	Date of emergence.	Last flies died.	Maximum longevity.	Temperature.		Remarks.
				Min.	Max.	
	1914.	1914.	*Days.*	°F.	°F.	
M–1.....	Oct. 18	Nov. 27	40	18	79	Minimum temperature of 18° F. occurred November 24.
M–2.....	Nov. 12	Dec. 14	32	18	67	
M–3.....	Nov. 15–17	Dec. 15	28–30	10	67	Minimum of 10° F. occurred on December 15, 1914, a. m. All appeared dead but were kept in insectary till Feb. 12, 1915, then taken to greenhouse, but none recovered after several days in a warm room.
M–7.....	Nov. 27	Dec. 15	18	10	64	Minimum temperature of 10° F. occurred December 15, 1914, a.m. No recovery after several hours in warm greenhouse.
M–8.....	Nov. 27	Dec. 15	18	10	64	
3–A.....	Nov. 6	Dec. 4	28	20	73	
4–A2.....	Nov. 3	Dec. 4	32	18	79	
5–A1.....	Nov. 2	Nov. 21	19	20	79	
5–A2.....	Nov. 12	Nov. 30	18	20	67	
5–A3.....	Nov. 2	Dec. 1	29	18	79	
5–A4.....	Nov. 7	Dec. 4	27	18	73	
11–1.....	Nov. 11	Dec. 14	32	18	64	
	1915.	1915.				
M–20.....	Mar. 2	Mar. 13	11	23	53	Reared in greenhouse and given time for one feeding before being taken outdoors. Continued cold prevented any feeding after removal to insectary.

In these and other experiments it was found impossible to keep house flies alive during the winter in cages under outdoor conditions in spite of the presence of food and shelter afforded by the mass of food material and the corners of the wooden framework of the cages. It was found that the first really cold night of the winter (December 15), when the temperature fell as low as 10° F., proved fatal. No flies revived after exposure to this temperature even when kept several hours or even days in a warm greenhouse. On the other hand, after exposure to temperatures of 22° or 25°, a large percentage often revived. Continued exposure to low temperatures which interfere with normal activities, especially feeding, will eventually prove fatal, as was the case in experiment M–20, Table I. Since at this latitude temperatures often fall below 10° during the winter, the foregoing experiments point to the conclusion that house flies in the adult state can not pass through the winter when exposed to outdoor conditions.

IN PROTECTED OR SLIGHTLY HEATED LOCATIONS

An attempt was made to test experimentally the prevailing idea that house flies can pass the winter in cracks and crevices in protected places of houses and stables. A portion of one of the barns of the Arlington farm was selected. The barn is a two-story structure, the first floor of which has stalls for 25 horses, a carriage room, and harness room. On the second floor are storage spaces for hay and grain, and at one end over the carriage room is the farm office. Steam pipes conducting heat to this office pass under the concrete floor of the carriage room and up inside the wall of the harness room. The carriage room has large doors on the south side and three windows on the north. A certain amount of heat is given off from the steam pipes in the harness room and through the floor so that the temperature is modified and does not fluctuate as much or as rapidly as does the outdoor temperature. A shelf was constructed in the carriage room about 5 feet from the floor and some distance away from both the steam pipes and the windows. Fly cages were kept on this shelf, and a thermograph was installed.

Of several experiments conducted here the following two gave the best results:

EXPERIMENT No. 13.—Sixty-five flies (45 males and 22 females), which had been reared in the greenhouse, emerged between December 26 and December 28, 1914. They were transferred to wire cages and supplied with banana and a mixture of bran and fresh horse manure well moistened. This material was renewed from time to time as needed. The cage was put on the shelf in the stable on December 29 and was examined every other day after this date. The flies died, a few at a time, up to March 9, 1915, when the last ones (9 males and 8 females) were found dead. In this experiment, then, 17 flies had been kept alive for a period of from 70 to 72 days. The maximum temperature during this period was 62° and the minimum was 29°. The mean temperature for the entire period was 43.8°.

EXPERIMENT No. 16.—From rearing experiments in the greenhouse 46 flies emerged between January 28 and February 3, 1915. These were transferred to a cage with the same kind of food as in Experiment No. 13, and placed on the shelf in the stable on February 3. The last fly of this lot, a male, was found dead on March 9, 1915, giving a maximum longevity of from 41 to 47 days. They were subjected to the same temperature conditions as obtained in experiment 13.

The following season, experiments of a similar kind were conducted in the attic of the apicultural laboratory of the Bureau of Entomology at Drummond, Maryland. This attic has one large south room which is plastered and heated. The heat was turned off and the door communicating with the unplastered part was left open. Cages were kept on a table in this room and the flies were supplied with banana and a sponge soaked in a sugar solution. Maximum and minimum thermometers were used in recording temperatures.

The best results were obtained in experiments 60 and 61 following:

EXPERIMENT No. 60.—House-fly puparia were collected on November 11, 1915. Twenty flies, emerged between November 13 and 16, were transferred to a cage and

placed on the table in the attic. Fresh food was supplied as needed, and a daily watch was kept. The last flies of this lot died on January 4, 1916, thus living from 49 to 52 days.

EXPERIMENT No. 61.—A similar experiment was conducted with flies which had emerged on November 17 to 19, 1915. The last flies died on January 10, 1916. This gives a longevity of from 52 to 54 days. The maximum temperature during this period was 76°, the minimum 35°, and the mean, 56.9°.

These experiments with caged flies in protected or slightly heated locations gave longevity records of 41, 47, 49, 52, 54, and 70 days. Dove (5) [1] records an experiment at Dallas, Texas, in which a fly was kept alive for 91 days in a large cage kept in an unoccupied room. In the latitude of Washington it would be necessary for flies to live at least from the first week of December (the time of the last emergence in the fall) until early in April (when temperatures first are high enough to induce oviposition) and be in condition to oviposit at the end of that time.

IN HEATED BUILDINGS

Overwintering experiments were also carried out in one of the warmest rooms of the greenhouses at the Arlington Experimental Farm. The room used was one in which tomatoes were grown, with temperatures ranging as a rule from a minimum of 55° or 60° at night to about 80° during the day. The humidity also was usually high. A few lots of flies reared in experiments during the fall of 1914 were transferred to this greenhouse at times during October and November. These flies were kept in cages already described and supplied with banana and fresh horse manure or a mixture of bran and horse manure. Eggs were readily obtained except when the fungous disease *Empusa muscae* killed off the adults too rapidly, and from the original lots of flies several generations were reared during the course of the winter. Table II gives the principal results of the experiments bearing upon the question of how long the flies will live in heated rooms during the winter.

In these experiments, where caged house flies were kept in a warm humid atmosphere during the winter, longevity records of from 9 to 40 days were obtained. It will be noted from Table II that the short records are all due to the attacks of *Empusa muscae*. There is, however, no evidence that adult flies, even if they escape the attacks of the fungus, can survive the winter in heated buildings. They were kept alive much longer in places such as attics and stables, which were only slightly heated.

[1] Reference is made by number to "Literature cited" pp. 168–169.

TABLE II.—*Longevity of house flies during the winter, in a heated room in a greenhouse*

Experiment No.	Flies emerged.	Last fly died.	Maximum longevity.	Remarks.
	1914.	1914.	*Days.*	
M-4.....	Nov. 18	Nov. 29	11	Fungus appeared on sixth day after emergence, and killed all flies.
M-5.....	Nov. 19	Nov. 30	11	Two dead on third day. Fungus appeared first on eighth day after emergence, and killed all remaining flies.
M-6.....	Nov. 21	Dec. 4	13	Fungus first appeared on sixth day after emergence.
M-8.....	Nov. 28	Dec. 12	14	Fungus appeared first on sixth day after emergence, and was cause of all deaths.
M-11...	Dec. 2	Dec. 15	13	Fungus appeared on eighth day and caused all subsequent deaths.
		1915.		
M-14...	Dec. 30	Feb. 8	40	No deaths from fungus, although the cage had previously held diseased flies and had not been disinfected.
	1915.			
M-17...	Feb. 7	Mar. 1	22	No fungus noted.
M-18...	Feb. 26	Mar. 20	20	Do.
M-21...	Mar. 3	Mar. 31	28	Do.
5-A5...	Nov. 7	Nov. 23	16	Fungus first appeared on tenth day, and was cause of all deaths.
5-A6...	Dec. 4	Dec. 18	14	Fungus appeared on the sixth day and was cause of all deaths.
5-B1...	Dec. 9	Dec. 28	19	Fungus appeared on the fourteenth day.
5-B2...	Dec. 13	Dec. 31	18	Fungus appeared on the tenth day.
5-B3...	Dec. 16	Dec. 31	15	No fungus noted.
11-A1..	Nov. 25	Dec. 4	9	Severe fungus attack, beginning on sixth day after emergence, was cause of all deaths.

Incidentally the experience with *Empusa muscae* in these experiments brought out the following points: (1) All the flies which emerged before December 30, with one exception (see lot No. 5-B3, Table II) were attacked by the fungus. All the lots of flies which emerged after December 30 gave no evidence of fungus attacks, although kept under the same conditions in cages which, earlier in the year, had held diseased flies, and which subsequently had not been disinfected. (2) The shortest time between the emergence of the flies and the first death from *Empusa muscae* was 6 days, but all were not affected at the same time; in some cases the period being extended to 19 days. It was found possible to obtain eggs from nearly every lot of flies before the fungus finally destroyed them. Even if it were possible to propagate *Empusa muscae* artificially and to disseminate the spores where they would be taken up by flies, it is doubtful whether it would be entirely effective, since, as the records show, eggs were often obtained before all the flies had been killed. (3) Experiments with the house fly do not support Dove's theory, based on experiments with Lucilia, that the fungus develops principally in sexually mature and in fertilized flies, which do not oviposit on account of low temperatures or in the absence of media for deposition.

OVERWINTERING OF ADULT FLIES UNDER NATURAL CONDITIONS

Frequent observations were made during the winter on the occurrence and behavior of house flies found living under natural conditions. For convenience in comparison with the experimental results these notes are summarized under the same headings.

UNDER OUTDOOR CONDITIONS

In the fall of 1914 observations were begun early in November. Adult house flies were found outdoors during warm days up to December 9. On December 1 several house flies were collected at a compost heap on the Arlington farm, and again on December 3. On this same date several recently emerged flies were taken near a heap of pig manure at the Bethesda farm. On December 9 many recently emerged flies were found on a heap of horse manure at College Park, Md., but after this date none were found outdoors until April, 1915, when the following notes were made:

April 7. No *Musca domestica* taken. Collected *Pollenia rudis* Fabricius, *Phorbia cinerella* Fallén, Sepsidae, and Borboridae.

April 8. Swept from horse dropping in road *Lucilia sericata* Meigen, *Orthellia cornicina* Fabricius, *Myospila meditabunda* Fabricius, *Phorbia cinerella* Fallén, *Copromyza equina* Fallén, and an undetermined species of Scatophaga.

April 10. Air temperature, 80° F., at 2 p. m. Search was made at pigpens, stables, manure heaps, etc. No *Musca domestica* found. *Muscina stabulans* Fallén was collected.

April 16. No *Musca domestica* found. Collected *Pollenia rudis*, *Phormia regina* Meigen, *Lucilia sericata*, and others.

April 25. A few house flies were taken by Max Kisliuk near stable at College Park, Md.

April 30. One female house fly taken near stable at the Arlington farm.

During the winter of 1915–16 flies were found outdoors as late as December 4. On that date one house fly was seen on wall on the south side of one of the animal houses of the Bethesda Experiment Station. No house flies were again found outside until the following April. Several flytraps were set out at various places on the Bethesda station, some on March 30 and others April 6, and were kept in operation throughout the season. Many species were caught, including *Pollenia rudis*, *Phormia regina*, *Muscina stabulans* Fallén, and *M. assimilis* Fallén, from the beginning of the exposures, but *Musca domestica* put in its first appearance on April 20, 1916.

The records for the winter of 1916–17 are similar. House flies were collected as late as December 12, 1916, after which none were again taken until the following spring. A few house flies put in an appearance at a very early date this year. Two females and one male were taken in a flytrap on March 26, 1917. A few scattered specimens were taken at various times during April. The unusually early appearance of house flies outdoors at this date was doubtless due to the fact that breeding

had continued and flies were present all winter in the animal house (see pp. 156–157) and that they escaped from the house on mild days during March and early April.

It will be noted that these observations on wild flies under outdoor conditions agree very closely with the experimental results with caged flies. Uncaged flies disappeared at about the same time that the caged flies were killed by the cold in early December.

UNHEATED BUT PROTECTED SITUATIONS

A very favorable unheated but protected location was found in one of the stables of the Arlington farm. This stable was at the west end of a long building and was protected on the north and east by a stone wall and high ground. The entrance was at the west and the ceiling was low and tightly boarded. Flies were collected at frequent intervals as late as January 19, 1915. Many of these were found with the ptilinum still exserted, showing that they were freshly emerged. Puparia were found in an accumulation of material under one of the feed troughs, and from them flies were emerging. All flies completely disappeared, however, after January 19 and none were again taken here until April 30, 1915.

At Bethesda, Md., no house flies were taken in unheated stables from December 7, 1915, till April 27, 1916. During the winter of 1914–15 collections were made in the attic of a dwelling house on the Arlington farm, and during the next two winters a careful watch was kept in the attics of one or two dwelling houses and of the apicultural laboratory at Drummond, Md. No house flies were found in such locations, although *Pollenia rudis* was present throughout the entire winter each season.

The experimental results showed that attics and slightly heated stables and similar places offer the most favorable temperature conditions conducive to longevity. Yet observations indicate that house flies never select such places for spending the winter. The records given by Copeman and Austen (*4*) of flies sent in to them from various places in England include no specimens of house flies taken in dormant or inactive condition in attics or other unheated places. It is shown that they "were all taken in an active condition" and from heated locations, such as dining rooms, kitchens, or bake shops.

IN HEATED BUILDINGS

Copeman and Austen (*4*) pointed out that—

> under the exceptionally favorable conditions afforded by confectioners' shops and restaurants, house flies of both sexes may survive in some numbers, at any rate until February.

But there is—

> nothing in the shape of proof that female house flies found alive at the end of the winter actually survive until oviposition takes place. * * * The ultimate fate of these insects is however at present unknown.

By restricting observations to certain selected places and by continuing them throughout the entire season the writer has been able to collect data which, it is believed, offer an explanation of the presence of flies during the winter months and of their ultimate fate. The following paragraphs bear on this point.

Some observations were made at a boarding house near Washington during the winter of 1915–16. There seemed to be complete indifference on the part of the occupants to the presence of flies, and the writer, in order to make some observations on these flies under natural conditions, was careful not to arouse any enthusiasm for fly killing. House flies were present in kitchen and dining room, and occasionally in bedrooms, throughout the fall and early winter. Their numbers gradually decreased as winter set in, but a few stragglers were noted and watched well into January. The last of them (two males) were noted in bedrooms on January 31. None were again seen in this house until the following May, some time after they had first appeared outdoors.

At the Bethesda station a small building is used daily for baking a coarse bread from meat scraps and corn meal to be fed to dogs. Temperatures were always high during the day, but the fires were allowed to go out during the night, and at such times the temperature often fell below freezing. House flies were present in large numbers during the fall. *Empusa muscae* carried off large numbers of them, but, in spite of this, some persisted during December and well on into January. There was a gradual and steady decrease and the last ones were observed on January 27, after which none were again seen here until the following May.

In both the above cases the flies had free access to food, but there was hardly any opportunity for oviposition. Their behavior was quite normal—that is, when the buildings were heated, they were active, invading everything with their usual annoying persistence, and when the temperatures were low they could be found at rest on the ceiling or walls, especially in the corners.

During the winter of 1916–17 special attention was paid to the conditions existing in the animal house at Bethesda, where large numbers of rabbits and guinea pigs are kept. The building used for this purpose is a long two-story structure, the walls of the first story being of masonry and the second story of frame construction. The floor of the first story is below ground level, and on this floor near the north wall is located the heater of the steam-heating plant. The temperature is maintained at about 60° F. throughout the winter, but in the immediate vicinity of the heater the temperature is, of course, considerably higher. From time to time cabbages were brought in from the storage banks and piled on the floor between the heater and the wall until used for feeding. A certain amount of decay always set in, especially in some of the outer leaves of the cabbages which had previously been slightly frozen. The

warmth from the heater and the odor of decaying cabbage proved very attractive to house flies, and large numbers congregated here early in the fall and were present in considerable numbers throughout the winter. That the house flies present here during March and the early part of April, 1917, were not the survivors but rather the offspring of those which had congregated here in the fall of 1916 is quite clearly indicated from the following observations:

It was noted that large numbers of these flies were carried off during December and early January by the attacks of a fungus, doubtless *Empusa muscae*. For example, on January 4, on one of the supporting posts near the heater, 68 victims of the fungus were counted. These were swept off, and six days later about 30 fresh victims were counted. Shortly after this date all the woodwork of the interior of the building was painted and no more deaths from fungus were noted, but dead flies were occasionally found on the floor below windows or between the windows and screens. In spite of these deaths there was no very noticeable falling off in the number of active adults which frequented the pile of cabbages. It was evident that breeding was taking place and that freshly emerged flies were steadily appearing to take the place of those killed by natural causes.

The breeding places were not definitely located. The cages for rabbits and guinea pigs were kept in excellent condition. All excrement, soiled litter, and waste food were removed from the cages every day, or at least every other day, and hauled away from the building. Floors under and around the cages were swept clean. The only possible place for the development of the larvæ seemed to be in the decaying portions of the cabbage. No lot of cabbage remained on the floor near the heater more than four or five days before it was used for feeding. It is possible, however, that flies may have oviposited on some decaying portion of the cabbage, and the issuing larvæ may have had three or four days development before being disturbed. Then, some may have been transferred with the food to the animal cages, where they possibly would have had time to complete their growth and to pupate in some obscure corner of the cage or in clean litter, and thus escape the cleaning process. The warmth near the heater and the warmth in the cages from the bodies of the animals would be sufficient to carry through development to pupation at about the normal rate. A study of the adults showed conclusively, however, that breeding was taking place and that freshly emerged flies were appearing from time to time.

Deposition of eggs was easily induced when a suitable medium was exposed. Thus, on December 13 a 1-gallon, wide-mouthed glass jar was half filled with moistened bran and exposed on a low support near the pile of cabbages. The warmth soon produced an active fermentation and eggs were obtained on December 15. Again, similar depositions were obtained on December 22, December 28, January 6, and other sub-

sequent exposures. In no case was there failure to obtain eggs within two or three days after exposure of the moist bran. The eggs so obtained were, of course, removed from the building in order not to interfere with the normal course of events.

The foregoing observations on deposition were supplemented by a study of a number of females captured from time to time during the winter. They were first examined to determine whether or not they were freshly emerged. This is done by exerting a little pressure on the sides of the thorax with a pair of forceps. In the case of very young flies this causes the ptilinum to extrude, even if it had previously been completely retracted. In the course of some other experiments it was found that in flies exposed to rather low temperatures the ptilinum may retain this elasticity for as long as 12 days, but at the temperature of this animal house it is probable that the ptilinum can not be forced out after the flies are three or four days old. When such pressure does not cause the ptilinum to extrude, it is certain that the fly is past this early stage of plasticity, but how much older it may be is, of course, unknown.

After this examination the flies were dissected and the spermathecæ were examined for the presence or absence of spermatozoa, and the size and development of the ovaries was studied. A regular procedure was adopted in this study, as follows: The abdomen was removed from the freshly killed fly, and from this the entire reproductive system was removed. This was put on a hollow ground-glass slide and covered with physiological salt solution. The spermathecæ were then removed and mounted on a plain glass slide in salt solution and examined under the compound microscope (4-mm. objective). A little pressure on the cover glass will cause the chitinous capsule to break open and the presence or absence of spermatozoa is easily determined. Plate 7, figure G, gives an idea of the appearance of the spermathecæ just after they have been thus broken open. The spermatozoa in lively wriggling masses are seen issuing from the cracks of the capsule walls and also from the severed ends of the ducts. The lines of the figure which represent the spermatozoa are relatively darker and heavier than they actually appear under the microscope.

The ovaries were fixed in Carnoy's fluid and washed in alcohol and then stained in carmalum or borax carmine. After destaining and dehydrating, the ovarioles were separated by careful manipulation with needles and mounted in balsam. Sometimes a little picric acid was added to the 95 per cent alcohol during the dehydrating process in order to bring out the chitinous structures more clearly. The ovaries were found in all stages of development. Camera-lucida drawings of certain well-marked stages were made. These are shown in Plate 7, figures A to F. They are drawn to the same scale, the No. 10 eyepiece and the 16-mm. objective being used. Most of the figures show the structures in optical section, but the surface markings have been represented

on some of the larger cells. These six stages were selected arbitrarily as standards of reference, and all the other ovaries were compared with them and grouped according to the stage which they most closely approached. The results of this study are given in Table III.

TABLE III.—*Showing presence of spermatozoa and the stage of development of the ovaries in house flies taken during the winter in a warm building, Bethesda, Md., 1917*

Date.	Number of females dissected.	Condition of spermathecæ.		Ptilinum extruded (freshly emerged flies).	Condition of ovarioles at developmental stages 1–6.					
		Spermatozoa present.	Spermatozoa absent.		1	2	3	4	5	6
1917.										
January 10	6	4	2	.....	[a] 1	[a] 1	2	.....	2	.....
January 15	2	2	.....	.....	.....	.....	.....	1	1	.....
January 17	4	4	.....	.....	.....	.....	2	1	1	.....
January 24	2	1	1	.....	.....	[a] 2	.....	.....	.....	.....
January 30	2	1	1	1	[ap.e.] 1	.....	.....	.....	.....	1
February 2	4	3	1	.....	.....	[a] 1	1	.....	1	[b] 1
February 6	4	4	.....	.....	.....	.....	1	1	2	.....
February 8	4	2	2	.....	[a] 2	1	.....	.....	1	.....
February 12	3	2	1	2	[ap.e.] 1	[p.e.] 1	1	.....	.....	.....
February 13	3	3	.....	.....	.....	1	2	.....	.....	.....
February 16	4	4	.....	.....	.....	.....	3	.....	1	.....
February 17	2	2	.....	.....	.....	.....	1	.....	1	.....
February 21	4	2	2	1	[ap.e.] 1	[a] 1	.....	.....	1	1
February 26	2	1	1	.....	.....	[a] 1	.....	.....	.....	[c] 1
March 9	4	3	1	.....	.....	1	.....	[a] 2	.....	[c] 1
March 13	3	2	1	.....	.....	[a] 1	2	.....	.....	.....
March 17	4	4	.....	.....	.....	.....	2	.....	2	.....
March 21	2	2	.....	.....	.....	.....	1	1	.....	.....
Total	59	46	13	4	6	11	18	6	13	5
Percentage of total	.....	78	22	6.7	10.1	18.6	30.5	10.1	22	8.4

[a] Indicates ovaries of flies from which spermatozoa were absent.
p.e. Indicates those in which ptilinum could be extruded.
[b] Two eggs lodged in oviduct showing that deposition had recently occurred.
[c] Mature egg lodged in oviduct, showing that deposition had recently occurred.

Table III shows that of the 59 females examined during the period from January 10 to March 21, 1917, 46, or 78 per cent, contained living spermatozoa in the spermathecæ, while in 13, or 22 per cent, the spermathecæ were empty. In 6 flies, or 10 per cent, the ovaries were in the first stage of development. Correlated with this was the entire absence of spermatozoa and the fact that in 3 of them a slight pressure on the thorax caused the ptilinum to extrude. This proves conclusively that they were very young flies. Of the 11 flies, or 18.6 per cent, with ovaries in the second stage of development, spermatozoa were found in only 3 cases, and the ptilinum could be extruded in 1 case. They were but slightly older than those of stage 1. With one exception (a fly with

ovaries in the fourth stage) all flies with ovaries past the second stage of development contained spermatozoa. In 13 flies, or 22 per cent, the ovaries were apparently mature, and eggs were ready for deposition. In 5 flies, or 8.4 per cent, the condition of the ovaries indicated clearly that deposition had occurred only a short time previous to the examination. The ovarioles had the appearance of figure F (Pl. 7), and in 3 cases mature eggs were seen lodged in the oviduct at the point where the oviducts unite to form the common oviduct.

A comparison of the foregoing findings with the conditions found in *Pollenia rudis* is very instructive. There is hardly any doubt that Pollenia hibernates in the adult state. It is found at all times throughout the winter in attics, unoccupied rooms, and the like, and is often seen outdoors in mild weather. Frequent collections were made during the winter of 1916–17, and the spermathecæ and ovaries were studied in the same way as those of the house fly. The spermathecæ were found to be empty in all cases examined during December, January, and February. The first spermatozoa were found in a female examined on March 22. Correlated with this was the fact that, without exception, all ovaries corresponded in development to stage 1 (Pl. 7, A) of the house fly. Not until early in March was any more advanced stage of development encountered. This agrees exactly with Kisliuk's observations (*13*) on Pollenia at Columbus, Ohio, where he found no development of the ovaries until early in March. Kisliuk (*13*) also succeeded in obtaining eggs from specimens of Pollenia captured on March 21. A specimen of Pollenia was kept alive in a cage in the screened insectary at Bethesda, Md., for a period of about four months during the winter, and during this time the temperature reached a minimum of −1° F. It appears, then, that those individuals of Pollenia emerging in October and November overwinter as adults, being active on warm days, but clustering in corners and hiding in cracks and crevices during cold periods. The ovaries remain undeveloped until spring. During warm periods in March the ovaries begin to develop, copulation takes place, and eggs are soon ready for deposition.

Now, if *Musca domestica* did hibernate as an adult, one would expect to find a similar suspension of sexual development even in warm buildings, and to find the reproductive power conserved to be exercised only in the spring when outdoor activities could be resumed. *M. domestica*, however, does not show any such retardation of ovarian development; nor does it display the same ability to withstand the effects of cold. Another important fact is that *M. domestica* was never collected during the winter in attics and other unused but protected places where Pollenia is found in such abundance, although experiments with caged house flies showed that the temperature of such locations was most favorable for the prolongation of life. In other words, adults of *M. domestica* were found during the winter only where food was available, and persisted

throughout the winter only where conditions permitted breeding to continue uninterruptedly.

Jepson (*12*) and other observers have proved experimentally that house flies will breed during the winter under the proper conditions. The foregoing studies on the development of the ovaries and the behavior of the flies in the animal house at the Bethesda station, and Kisliuk's observations (*13*) in the animal house at the Ohio State University and in the Columbus (Ohio) garbage disposal plant, prove that breeding does normally take place in locations where artificial heat, food material, and breeding media are available. Breeding continues all winter in such places, and then on warm days in the spring the flies escape through open windows or doors and take up their usual outdoor activities. This is very probably the explanation of the early appearance of those flies taken in traps on the Bethesda farm in March, 1917, and also of those found by Dr. Henry Skinner (*17*) on March 3, 1913, since in a city there are doubtless many places where breeding occurs throughout the winter.

RELATION OF TEMPERATURE TO ACTIVITY

The behavior of house flies at ordinary summer temperatures has been treated fully in the works of L. O. Howard (*11*), Hewitt (*8*, *9*), Graham-Smith (*7*), and others. But the following brief notes on the effect of low temperatures on activity have a direct bearing on the question of overwintering. Observations were made on both laboratory-reared caged flies and wild flies:

At temperature of

- 65° F. . Flies are quite active.
- 55° F. . Flies are fairly active. Copulation was noted at this temperature, but no sexual activity has been noted at lower points.
- 52° F. . Flies only slightly active.
- 42° F. . Flies move rather weakly when disturbed, but are otherwise inactive. In cages they are usually found on the sides or in corners near the top.
- 40° F. . Flies inactive; crawl feebly when touched.
- 34° F. . Flies inactive; may move legs in weak uncoordinated way when touched.
- 30° F. . One male survived exposure for one week at this temperature. (*10*).
- 29° F. . Usually no reaction when touched, but they quickly revive when brought into a warm room.
- 25° F. . Appear to be dead, but most of them revive when brought into a warm room.
- 22° F. . Apparently dead, but in some cage experiments it was found that a considerable percentage revived when warmed after exposure to this minimum during the preceding night.
- 12° F. . All dead; no recovery when taken to a warm room. "None survived exposure for one week." (*10*).
- 10° F. . All dead.

The fatal temperature is thus shown to be quite low, and the fact that many revive after exposure to temperatures as low as 22° F. explains why flies persist outdoors in this latitude as late as the first week in December. The most important point is that flies will resume activities when the temperature is raised, even after exposure to rather low temperatures. There is no hibernation in the sense of long-continued suspension of activity during the winter months. Dove (5) states that he has never—

> observed living adults to remain quiescent for more than a few days. The natural heat of the sun or very slight artificial heat will cause them to become active.

OVERWINTERING OF LARVA AND PUPA STAGES

Probably every observer who has planned any work on this problem has started out with the assumption that "it might reasonably be thought that the pupæ would prove more resistant than the earlier stages" (3), and there have, in fact, been many attempts to carry the puparia of the house fly through the winter. "But," says Copeman, "so far as I am aware, no observer has succeeded in breeding out the flies from pupæ kept through an average winter in this country" [England]. "Nor," according to Hewitt's statement (9), "has it ever been possible in my breeding experiments in Canada and in England to carry the insect through the winter in the pupal state." Lyon (14) reports some experiments started on October 19, 1914, at Boston, Mass. Thirty-seven lots, of 100 puparia each, were buried in wet and dry sand, wet and dry loam, wet and dry horse manure, and leaf mold. These materials, with pupæ, were placed in glass jars, some in the basement of a stone building, some in an unused greenhouse, some in sheltered positions outdoors, and some in exposed positions outdoors. No flies emerged from those lots placed outdoors. Several hundred emerged from those kept in the greenhouse and in the basement, but even from these none emerged after December 1, 1914. Upon examination on June 23, 1915, the pupæ appeared normal, but on being broken open they were found to have completely dried out.

The writer has tried similar experiments and obtained similar results. About 1½ quarts of pig-manure-bran mixture containing approximately 900 puparia were collected at Bethesda, Md., on November 14, 1914. This mass was put in a large wooden box, covered with 3 or 4 inches of sand, and the box, covered with a plate of glass, was placed on a shelf of a screened insectary. House flies in considerable numbers emerged on mild days up to December 4. None emerged after that date. On March 17, 1915, the box was transferred to a warm room in a greenhouse and remained there until April 1, but no flies appeared. Another collection of pupæ made on December 3, 1914, and handled in the same way, also showed no emergence after December 4. In a third experiment special precautions were taken to prevent freezing, and pupæ of known

age were used. From rearing experiments in the greenhouse eggs were obtained January 9, 1915. These hatched and were carried through in horse manure confined in a large wooden box. From this lot about 150 puparia were collected on January 26, immediately placed in a box (8 by 8 by 10 inches), and covered with 4 inches of dry sand. The box was taken to the apicultural laboratory at Drummond, Md., and buried to within 1 inch of the top of the box in the ground near the east wall of the building. With the help of Mr. G. S. Demuth thermocouples were inserted in the sand among the puparia. The box was then covered with a glass plate and over this was placed a square of rubberized roofing and a large quantity of sawdust. Temperature records were taken eight times daily from this date until April 26, by means of the electrical apparatus used by Phillips and Demuth (*16*) in their work on the temperature of the bee cluster in winter. The actual temperature to which the puparia were exposed was uniformly low, but never less than 34° F. There was a gradual rise in temperature until 45° F. was reached, on April 26, when the readings were discontinued. On May 17 the box was taken from the ground. No flies had emerged. The puparia were collected and examined. While their external appearance was normal, of 10 puparia which were dissected, 7 were found to contain a brown sticky liquid, and in 3 the pupæ, although well formed, were completely dried up. The other puparia were kept in ordinary garden soil until July 17, but no flies emerged.

All these experiments are open to the criticism that the conditions were more or less artificial, and that relatively small numbers of puparia were used. Attempts to provide more nearly natural conditions and to determine the possibility of overwintering in large heaps of manure were first reported by Bishop, Dove, and Parman (*2*). They report two cases in which they succeeded in carrying the house fly through the winter in the immature stages. In one of these, at Dallas, Tex., 3 barrels of horse manure, cow manure, and straw were put in a cage on November 26, 1913. No flies emerged after December 27 until April 16, 1914, when 4 specimens appeared. Others emerged May 26. Larvæ were found in some numbers up to March 21. Thus it was shown that the house fly lived in the larva stage for a period of 115 days, and in the larva and pupa stages until April 16 and May 26, periods of 141 to 181 days. In a second experiment, at Uvalde, Tex., horse manure and straw were put in a cage on December 6, 8, 9, and 13, 1913. Emergence continued during warm periods throughout the winter. From 1 to 9 flies emerged daily from March 1 to 18, and on each of three days (Apr. 1, 2, and 4) a single fly emerged. Dove (*5*) gives further details of these experiments.

The publication of the experiments of Bishopp, Dove, and Parman (*2*) brought up the question whether they were paralleled under more severe winter conditions, or whether they obtained only in the moderate climate of Texas and the Gulf coast. Experiments similar to these were carried

out by the writer at Bethesda, Md., during the two winters of 1915–16 and 1916–17, but not until the winter of 1916–17 was any evidence obtained which tended to show that overwintering in the larva and pupa stages was possible in the more northerly latitudes. The results of these experiments are here briefly reviewed.

Six wooden frames 7 feet square and 2 feet high were set in the ground to a depth of 6 inches. On these were placed pyramidal screened covers. The pyramids were slightly truncated and provided with a 6-inch opening at the top, over which a large flytrap was fastened. The whole was carefully fitted, so that there was no escape for flies except into the trap at the top. Migrating larvæ would have to burrow at least 6 inches into the soil in order to escape under the wooden sides of the cages. From October 22 to 29, 1915, 8 bushels of fresh horse manure and straw were put in each box. To this was added about 5 quarts of pig-manure-bran mixture, which had been collected from a heap near some pigpens and which contained thousands of larvæ in different stages. Additions of both horse manure and pig manure were made at intervals until December 8, 1915, when there was a total of about 25 bushels. The pyramidal covers, with flytraps attached, were put in place on November 4, and records of emergence from three cages were kept during the fall and of all six cages during the following spring.

From the three cages records of which were kept during the fall emergence ceased after December 3, 1915. The number and species are given in Table IV.

TABLE IV.—*Number and species of flies emerged from three heaps of horse and pig manure from November 4 to December 3, 1915*

Species.	Total number of flies emerged.	Species.	Total number of flies emerged.
Musca domestica	1,062	*Muscina assimilis*	1
Stomoxys calcitrans Linnaeus	242	*Orthellia cornicina*	1
Morellia micans Macquart	2	*Phormia regina*	1

During the following spring emergence began April 6 and continued as late as June 1, 1916. The records for all six heaps are given in Table V.

In these experiments *Musca domestica* was found emerging up to December 3, 1915, but none were taken after this date, although the heaps were kept under observation until June 1, 1916. The same is true of *Stomoxys calcitrans*. Of the six species taken during the fall only one (*Muscina assimilis*) appeared again in the spring in these traps. Eight species in addition to the three or four species of the Sarcophagidae were taken during the spring, and thus proved to have overwintered in the larva and pupa stages. Of these *Hydrotaea houghi* and *Ophyra leucostoma*

are not normally found in horse manure, but were doubtless introduced in the pig-manure-bran mixture.

TABLE V.—*Number and species of flies emerged during the spring of 1916 from six heaps of overwintered horse and pig manure*

Species.	Total number of flies emerged.	Period of emergence.
Muscina assimilis	8	May 4 to 20.
Muscina stabulans	1	May 4.
Fannia canicularis Linnaeus	35	April 6 to May 22.
Hydrotaea houghi Malloch	237	April 24 to May 31.
Ophyra leucostoma Wiedemann	594	April 28 to May 31.
Phorbia sp	4	April 28 to May 4.
Scatophaga furcata Say	26	April 6 to May 6.
Syritta pipiens Linnaeus	3	May 6 to 22.
Sarcophagidae	18	May 2 to 29.

Three experiments were carried out during the winter of 1916–17. They were started on October 3, 1916, by placing in each of three wooden frames 8 bushels of fresh horse manure containing little straw and seen to be quite heavily infested with larvæ of both *Musca domestica* and *Stomoxys calcitrans*. Additions of horse manure were made from time to time up to January 8, 1917, when each heap contained 40 bushels of manure. Larvæ of *Musca domestica* and *Stomoxys calcitrans* were seen in all manure added to the heaps up to and including that of October 24, all additions after this date appearing to be free from infestation.

The first of these three experiments was managed somewhat differently from the others. On October 9, 1916, 1 quart of moist bran containing hundreds of house-fly larvæ was put on the heap. Again on three occasions—December 27, 1916, and January 4 and 10, 1917—2 quarts of moist bran which had been previously exposed in the animal house and was heavily infested with fly larvæ were buried in the heap. In this way fully 2,500 house-fly larvæ were added to the normal infestation of the manure. The pyramidal cover, with attached flytrap, was put in place on November 13. From this date to December 11 the following species were taken from the trap:

Musca domestica	20	*Fannia canicularis*	1
Stomoxys calcitrans	247	*Scatophaga stercoraria*	2

No further emergence was noted during the winter until March 22, 1917. The trap was regularly emptied from that time until June 4, the catches being summed up in Table VI.

TABLE VI.—*Total number of flies emerged during the spring of 1917 from one heap of horse manure to which infested bran was added*

Species.	Total number of flies emerged.	Period of emergence.
Musca domestica	1	April 30.
Muscina assimilis	1	April 16 to 23.
Muscina stabulans	1	May 14 to 21.
Ophyra leucostoma	18	April 9 to 30.
Hydrotaea armipes Fallén	36	April 9 to May 14.
Fannia canicularis	39	April 16 to May 21.
Other species of Anthomyiidae	22	April 16 to 30.
Scatophaga stercoraria	26	March 22 to April 30.
Scatophaga furcata	145	April 9 to May 14.

The single specimen of *Musca domestica* taken from the trap on April 30 was plainly freshly emerged, having bright colors, soft body, and ptilinum not fully retracted.

Two other experiments were conducted in the same way as the one just described except that no bran with larvæ was added at any time, and therefore no larvæ were introduced after October 24. The manure added after this date was not infested. From November 13 to December 11, 1916, the following flies emerged from these heaps:

Musca domestica	34
Stomoxys calcitrans	200
Scatophaga stercoraria	1

No emergence took place from December 11 to April 2. From April 2 to June 4, a total of 347 flies were taken, as shown in Table VII.

TABLE VII.—*Total number of flies emerged during the spring of 1917 from two heaps of over-wintered horse manure*

Species.	Total number of flies emerged.	Period of emergence.
Musca domestica	1	April 23.
Stomoxys calcitrans	1	May 14.
Myospila meditabunda	3	April 20 to May 14.
Muscina assimilis	2	April 30 to June 1.
Fannia canicularis	33	April 23 to May 21.
Hydrotaea armipes	76	April 23 to May 25.
Other species of *Anthomyiidae*	50	April 16 to May 14.
Scatophaga furcata	158	April 2 to May 14.
Scatophaga stercoraria	23	April 2 to May 21.
Total	347	

From these three experiments only two specimens of house flies emerged during the spring. If this number is too small to prove this to

be the usual method of overwintering, at least it proves the possibility in the latitude of Washington. From a mass of pupæ collected in a pile of rabbit and guinea-pig manure on February 26, Kisliuk (*13*) was successful in rearing four house flies on March 10 and 12. McDowell and Eastwood (*15*) record observations of the same kind. Whether the house fly can overwinter in the larva and pupa stages in more severe climates is still doubtful. C. W. Howard (*10*) concludes from his observations that—

> the temperature of Minnesota winters is not favorable to the overwintering of the house fly in any except the adult stage and that stage only in places where there is a sufficiently high temperature and where food conditions are favorable.

SUMMARY AND CONCLUSIONS.

The present status of our knowledge of the overwintering habits of the house fly seems to the writer to warrant the following conclusions:

In the latitude of Washington, D. C., the house fly may overwinter in two ways: (1) By continued breeding in warm places where food and media for deposition are available, and (2) in the larva and pupa stages in or under large manure heaps.

There is no evidence whatever to show that house flies do or can persist as adults from November to April, either outdoors, in protected stables, or in attics or heated buildings. Temperatures of 12° or 15° F. are quickly fatal, and there is every reason to believe that any temperature below freezing is fatal if continued long enough. In heated buildings their life is not prolonged beyond that of summer at like temperature, nor is there any suspension or retardation of sexual development or activity.

It is known that house flies continue to emerge from manure heaps as late as the first week in December. Many of these late forms will find their way on mild days to heated buildings, and those which do not are quickly killed. Those that find their way to heated buildings are attracted, as in summer, to odors of food and will congregate in kitchens, dining rooms, restaurants, bakeries, animal houses, and the like. If no food is at hand they will quickly perish. When food is available they may continue alive through December and January and even into February, if not destroyed by fungus attacks. But there are neither experiments nor observations to show that they can continue throughout the winter until temperatures are again favorable for outdoor activity and egg laying. If flies find access in the autumn to heated buildings, where both food and media for deposition are available, such as animal houses or restaurants in which sufficient attention is not given to the disposal of garbage or kitchen wastes, they will continue breeding throughout the winter. In such cases the flies present in March and April are the offspring, not the survivors, of those which found their way to such places the preceding autumn. It is probable that this method of over-

wintering is much more widespread than is now realized, especially in cities where there must be several foci from which flies escaping on warm days in March and April, survive to produce the hordes that begin to appear late in May.

The possibility of house flies overwintering in the larva and pupa stages has been demonstrated at Washington, D. C., and at Columbus, Ohio, as well as for the milder regions of Texas. But whether this method of overwintering in these stages or by continued breeding is the more common or more successful can not now be stated. To judge from experiments with larvæ and pupæ and from the fact that house flies do not appear in large numbers until late in May or early in June it would seem that only a very small percentage of larvæ, which are present in manure heaps in the autumn, live through the winter and give rise to the adults in the spring.

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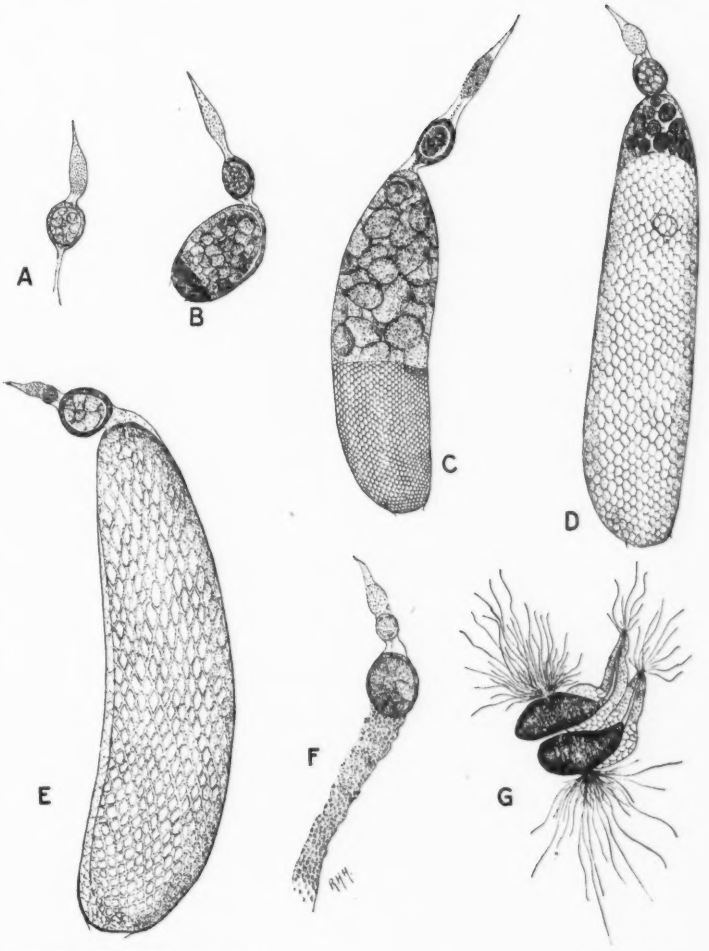
PLATE 13

Musca domestica:

A–F.—Various stages in the development of the ovarioles from the time of emergence of the fly until after deposition of eggs. Camera-lucida drawings from ovarioles stained in carmalum or borax carmine. All drawn on same scale. No. 10 eyepiece and 16-mm. objective.

G.—Two spermathecæ. Camera-lucida drawing from fresh preparation showing spermatozoa escaping from the severed ends of the ducts and from the splits in the sides of the capsules caused by pressure on the cover glass.


A
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SOIL ACIDITY AS INFLUENCED BY GREEN MANURES

By J. W. WHITE

Associate Professor of Experimental Agronomy, The Pennsylvania State College Agricultural Experiment Station

INTRODUCTION

The study of the general fertilizer series of plots at the Pennsylvania Experiment Station, which have been continuously treated according to different systems of fertilizing ever since 1881, has shown some instances of large lime requirements, particularly in the case of the soils of plots 30, 31, and 32, treated, respectively, with a complete fertilizer containing different quantities of nitrogen supplied in the form of ammonium sulphate. Experiments reported in recent years have shown clearly by the ordinary tests, that these soils are distinctly acid, that the common rotation crops fall off in yields in proportion to the degree of acidity of these soils, and that the use of either lime or finely ground limestone in quantity sufficient to make the lands neutral is all that is necessary to bring them back to normal bearing.

One of the questions raised by the facts just stated concerns the cause or causes producing the sour or lime-deficient conditions here observed. Among these causes commonly regarded as operative to set up an acid condition, or one of pronounced lime requirement in a soil, is the accumulation in it of a large amount of organic matter decaying under conditions or by the action of agents that form acid materials from it. On all cultivated soils, sods and crop residues are customarily plowed under and might serve as the raw material for the production of acid substances and, indeed, may have at the outset a direct acid effect, because they themselves contain, especially in the immature condition, considerable quantities of acid-acting constituents.

Doubtless, however, the heavy cover crops and intercrops, turned under as green manures, should most strikingly show the kinds of effects here in question. Practical experience has sometimes shown temporary ill effects to follow the turning under of especially rank growths of green manures, and their stage of maturity and their kind are regarded as having something to do with the appearance or nonappearance of these ill effects. This particular phase of the green manure subject has not to our knowledge, been elsewhere studied.

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MANURES USED

In our study of organic crop residue as possibly contributory to the development of acidity in these ammonium-sulphate plots we have used, as the organic materials to be mixed with the soil, various green-manuring plants, leguminous and nonleguminous, and some common weeds and less desirable grasses. These have been used both fresh and air dried with the thought that in the latter condition, while the plant materials would not have the exact balance of composition possessed by the corresponding mature growths, they would at least resemble the mature plants in physical quality and resistance to the bacterial penetrations. To the list of organic materials used (Table I), barnyard and poultry manures were added to ascertain what differences of behavior they might develop, with the view of thus more sharply distinguishing the real factors.

TABLE I.—*Green manures used in experiments*

Series I (applied green).	Series II (applied air-dried).
Legumes:	Legumes:
Soy bean (*Soja max*).	Soybean.
Canada field pea (*Pisum sativum*).	Sweet clover.
Sweet clover (*Melilotus alba*).	Alfalfa.
Alfalfa (*Medicago sativa*).	Red clover.
Red clover (*Trifolium pratense*).	Hairy vetch.
Hairy vetch (*Vicia villosa*).	
Nonlegumes:	Nonlegumes:
Wheat (*Triticum* spp.).	Rape.
Rape (*Brassica napus*).	Corn.
Oats (*Avena sativa*).	Rye.
Corn (*Zea mays*).	Redtop.
Rye (*Secale cereale*).	Sorrel.
Timothy (*Phleum pratense*).	
Redtop (*Agrostis alba*).	
Sorrel (*Rumex Acetosella*).	

The wheat, rape, and soy beans used were young, tender plants. The wheat was obtained from a pot experiment, where the growth was about 1 foot high. The other materials were removed from the field on July 6, and they represented an average stage of maturity at that period.

The composition of the several manures as used in Series I, with respect to the point judged to be most important in the present study, is given in Table II.

TABLE II.—*Composition of green manures and others used in experiments*

Material.	Original moisture content.	In water-free substances.					Quantity of fresh material to yield 10 gm. of dry matter.
		Organic matter.	Ash.	Alkalinity of ash.[a]	Water-soluble acidity of manures.	Nitrogen.	
Legumes:	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*Gm.*
Soy bean	76. 7	78. 04	21. 96	6. 92	1. 70	2. 78	42. 9
Canada field pea	80. 7	88. 85	11. 15	7. 80	4. 02	3. 65	50. 5
Sweet clover	72. 5	92. 16	7. 84	5. 27	2. 90	3. 12	36. 3
Alfalfa	65. 8	91. 37	8. 63	7. 82	2. 13	2. 76	29. 2
Red clover	61. 6	92. 75	7. 25	5. 63	. 28	1. 94	26. 0
Hairy vetch	66. 4	93. 04	6. 96	3. 27	1. 91	2. 84	29. 8
Nonlegumes:							
Wheat	75. 2	89. 36	10. 64	6. 00	. 19	3. 56	40. 2
Rape	89. 1	80. 33	19. 67	10. 48	2. 88	4. 79	91. 7
Oats	83. 2	88. 46	11. 54	5. 35	8. 15	3. 08	59. 5
Corn	87. 9	91. 29	8. 71	4. 70	5. 92	2. 72	91. 7
Rye	56. 1	95. 40	4. 60	1. 27	3. 07	1. 33	22. 7
Timothy	60. 1	93. 76	6. 24	2. 32	. 23	1. 29	25. 0
Redtop	60. 2	93. 99	6. 01	1. 60	. 42	1. 07	25. 1
Sorrel	80. 5	90. 18	9. 82	3. 67	3. 01	1. 98	50. 2
Other manures:							
Poultry manure	64. 7	71. 38	28. 62	9. 65	(b)	3. 35	28. 3
Barnyard manure	74. 7	60. 83	39. 17	5. 52	(b)	1. 57	39. 5

[a] In terms of the calcium-carbonate equivalent. [b] Alkaline.

SOIL USED

With the view of detecting a change in the degree of acidity or lime requirement after the addition of the green manures, a soil already distinctly acid was chosen. This soil was taken from plot 32. The lime requirement of this soil, as shown by concordant duplicate determinations by the Veitch method, was equivalent to 4,644 pounds of calcium carbonate to the acre 7 inches (2,000,000 pounds). This quantity will hereafter in this paper be called the "limestone requirement." The soil employed had, before this determination, been freed from stones and roots by sifting through a screen with meshes of a diameter of 3 mm. Before use it was thoroughly mixed to make it uniform throughout.

PREPARATION OF GREEN MANURES AND SOIL MIXTURES

The fresh green manures were cut fine with scissors and then passed through a sausage mill until thoroughly subdivided. The water removed from the green residues during the process was carefully replaced. The fine product was used for the experiment of Series I. Other portions of the same materials were carefully air-dried and similarly subdivided for use in Series II.

Of the fresh materials thus prepared, the respective quantities stated in the last column of Table II as equivalent to 10 gm. of dry matter were

each thoroughly mixed with 500-gm. portions of the soil. The mixture thus represents an addition of 20 tons of green manurial dry matter to 2,000,000 pounds, or an acre 7-inch layer of soil.

Like mixtures of corresponding amounts of the air-dried manurial substances were used with 500-gm. portions of the soil from Series II.

SUBSEQUENT TREATMENT

The mixtures thus prepared were kept in large jars freely exposed to the air and sunshine for two months; thereafter, until the close of the experiment, in a well-ventilated room. The entire period extended from July 7, 1914, to April 7, 1915. The jars were weighed at frequent intervals, and the losses of weight made up by the addition of distilled water so as to keep the soil quite close to the optimum moisture content with which it was started, in order that, as respects the moisture present, the fermentative conditions might be more favorable.

A control sample of the original untreated soil was similarly kept during this period.

LIMESTONE-REQUIREMENT DETERMINATIONS

At periodic intervals the limestone requirements of these mixtures were determined. To obtain samples for this purpose, the entire mass of material was removed from the jar, thoroughly mixed, a sample sufficient for duplicate determinations withdrawn, and the remainder of the soil returned to its jar, and put as nearly as possible into the original condition of compactness. The determinations were made by the Veitch method, the same as for the original soil.

Unfortunately the determinations were not begun until two weeks after the mixtures were prepared and exposed in moist condition as above described. The changes in limestone requirement during this period of fermentation may, however, be approximated if we assume that in each case it corresponds to the sum of the limestone requirement of the soil and the calcium-carbonate equivalent of the water-soluble acids in the manurial material used—that is, the sum of the limestone requirement of the soil and the calcium carbonate equivalent of the water-soluble acids in 40,000 pounds of the manurial dry matter, the manurial requirement for 2,000,000 pounds of soil.

The sum thus obtained must be reduced to the equivalent for 2,000,000 pounds of the mixture, because all subsequent determinations are based upon that quantity of the mixtures.

If the colloidal substances in the plant materials are capable of absorbing lime from limewater so as to increase the apparent acidity of the materials, the theoretical limestone requirement thus calculated is, of course, too low.

SERIES I: FRESH GREEN MANURES

For each mixture of the soil with the fresh material (Series I) this theoretical original limestone requirement and the actual requirement determined at the several periods of the experiment are stated in Table III; the amounts and direction of change during the corresponding intervals, in Table IV.

The figures of Table IV show that all the mixtures exhibited very great differences in limestone requirement from time to time during the nine months through which these observations extended. The degrees of variation significant of real differences, as distinguished from such as are due to difficulties of sampling and analysis, are well established by comparison of the duplicate determinations made at each period and for each mixture from separate portions of the mixture or soil itself. The average difference between such duplicates corresponded to about 200 pounds of limestone per acre.

TABLE III.—*Limestone requirement of green manures (Series I)*

[Results expressed as pounds of limestone per 2,000,000 pounds of soil]

Material.	Original (theoretical).	Period 1 (2 weeks).	Period 2 (4 weeks).	Period 3 (3 months).	Period 4 (5 months).	Period 5 (7 months).	Period 6 (9 months).
Control soil	4,644	4,644	4,711	4,644	4,677	4,871	4,904
Legume mixtures:							
Soy bean	5,220	3,627	4,712	4,678	5,661	5,994	5,650
Canada field pea	6,136	3,593	4,104	4,238	5,865	6,163	5,960
Sweet clover	5,690	3,187	4,949	4,983	6,509	6,570	6,502
Alfalfa	5,388	2,915	4,136	3,695	5,153	5,210	4,847
Red clover	4,663	713	1,153	.....	4,848	5,040	4,135
Hairy vetch	5,302	2,882	4,949	4,305	5,865	6,062	4,542
Subaverage	5,400	2,819	4,000	3,650	5,650	5,840	5,273
Nonlegume mixtures:							
Wheat	4,627	3,492	4,644	5,153	7,594	7,493	7,182
Rape	5,682	4,305	4,949	5,017	6,068	6,366	6,298
Oats	7,749	3,525	5,051	5,152	6,068	5,892	6,163
Corn	6,874	3,932	5,457	5,525	6,544	7,182	6,876
Rye	5,757	3,254	4,543	3,831	4,958	4,813	4,508
Timothy	4,643	2,610	4,271	3,831	5,356	5,243	4,847
Redtop	4,718	2,848	4,983	3,288	4,712	5,790	4,474
Sorrel	5,733	1,729	2,949	2,237	3,797	4,813	4,101
Subaverage	5,723	3,212	4,606	4,254	5,637	5,949	5,556
Barnyard-manure mixture	(?)	3,187	3,865	3,797	4,678	5,549	4,575
Grand average	5,584	3,053	4,314	3,982	5,578	5,879	5,377

TABLE IV.—*Changes in the limestone requirement from period to period (Series I)*

[Results expressed as pounds of limestone per 2,000,000 pounds of soil]

Material.	Period 1 (first 2 weeks).	Period 2 (second 2 weeks).	Period 3 (second and third months).	Period 4 (fourth and fifth months).	Period 5 (sixth and seventh months).	Period 6 (eighth and ninth months).	Total in 9 months.
Control soil	0	67	−67	33	194	33	260
Legume mixtures:							
Soy bean	−1,593	1,085	−34	983	333	−344	450
Canada field pea	−2,543	511	134	1,627	298	−203	−176
Sweet clover	−2,503	1,762	34	1,526	61	−68	842
Alfalfa	−2,473	1,221	−441	1,458	57	−363	−541
Red clover	−3,950	440	−1,154	?4,848	192	−905	−528
Hairy vetch	−2,420	2,067	−645	1,560	197	−1,520	−760
Subaverage	−2,580	1,181	−351	2,000	190	−567	−119
Nonlegume mixtures:							
Wheat	−1,135	1,152	509	2,441	−101	−311	2,555
Rape	−1,377	644	68	1,051	298	−68	616
Oats	−4,224	1,526	101	916	−176	271	−1,586
Corn	−2,942	1,525	68	1,019	638	−306	2
Rye	−2,503	1,289	−712	1,127	−145	−305	−1,249
Timothy	−2,033	1,661	−440	1,525	−113	−396	204
Redtop	−1,870	2,135	−1,695	1,424	1,078	−1,316	−244
Sorrel	−4,004	1,220	−712	1,560	1,016	−712	−1,632
Subaverage	−2,511	1,394	−352	1,383	312	−393	−167
Barnyard-manure mixtures	?−1,457	678	−68	881	871	−974	? −69
Grand average	−2,468	1,261	−215	1,596	300	−501	−141

The strength of the limewater reagent used was carefully standardized at each period; and even had it not been, the great differences in limestone requirement of the manurial mixtures, as compared with the control soil at the successive times of examinations, would bar such reagent variations as an explanation of the facts here recorded.

It is strikingly shown that the direction in change of limestone requirement was not continuously the same, but that, while there were some rather marked individual differences, there was, on the whole, a surprising agreement among the substances of the respective groups in the directions, time, and degree of the changes in this property.

Particularly notable are (1) The great reduction in limestone requirement during the first two weeks, a decrease on the part of the manurial mixtures to a point far below that of the control soil, in which the requirement underwent no change at this time; (2) a great increase, both absolute and relative, in the control soil in its requirement during the following two weeks; (3) the comparatively slight changes during the second and third months, save in the case of the redtop mixture; (4) a second extensive increase in the requirement during the fourth and fifth months; (5) slight changes during the succeeding months,

except in the case of the redtop and sorrel mixture, with a majority of slight increases during the sixth and seventh months, and a slight but usually greater decrease during the next two months.

It is particularly notable that there was no difference in tendency at these periods among the legumes, nonlegumes, and stable manures.

The several averages of Table III show the net effects of the several additions upon the reaction of the acid soil used as a basis.

The control soil without manurial addition gradually but slightly increased in acidity after five months' exposure under the conditions of this experiment. The addition of either the acid green manures or of alkaline stable manure caused, within two weeks, a very great decrease in limestone requirement. This lowered requirement was very pronounced for a very short time, however; and in a month the requirement had returned nearly to the level of that for the control soil. A later phase, manifest after the third month, showed a greater acidity or lime requirement for the manurial mixtures than for the soil; and with a few exceptions this continued until the end of the experiment.

The average conditions of lime requirement throughout the experiment of the individual manurial substances, after the fermentations had well started, were as follows:

	Pounds per acre.		Pounds per acre.
Red clover	2,648	Hairy vetch	4,767
Sorrel	3,271	Canada field pea	4,987
Stable manure	4,275	Oats	5,308
Rye	4,317	Soybean	5,053
Alfalfa	4,326	Sweet clover	5,450
Redtop	4,349	Rape	5,500
Timothy	4,359	Corn	5,919
Control soil	4,741	Wheat	5,926

In general, red clover as a green manure was the best corrective of acidity, taking the whole period into account, and sorrel was the only green manure whose addition kept the soil, at all times after fermentation had started, below the level of that exhibited by the soil alone. On the other hand, wheat and corn increased the average lime requirement more than any other of the manurial substances tried.

CAUSES OF THE CHANGES IN LIMESTONE REQUIREMENT

When the causes to which the marked changes are due are considered, we may narrow the field of inquiry by these considerations:

(1) The periodicity of the changes is not a result of variation in the environment, in moisture relation, or in aeration, but is probably incident to successive series of fermentations with corresponding chemical products.

(2) The general similarity in the changes exhibited by all the manurial mixtures indicates that these changes are related to the constituents in which they are alike rather than to those in which they differ.

(3) The properties of the soil itself have no direct causal relation to these changes, since these changes are but slightly if at all manifest in the control soil. The changes are therefore related either to the substances possessed in common by the manures, or to other substances produced by reaction between the plant materials and the constituents of the soil.

CHANGES OF THE FIRST PERIOD

This 2-week period immediately succeeding the beginning of the experiment shows no change in the limestone requirement of the control soil, but a very marked reduction in that of all the manurial mixtures with the soil. This reduction has been so great as not only to destroy the immediate acid actions of the green manures but also a large part of those which the soil by itself exerts.

Coville, in his study of the reactions occurring during the formation of leaf mold, has outlined the causes of the changes manifest in that case. The same chain of effects, other than such as are due to leaching, are doubtless to be found in the case of the green manures.

The alkaline effects so promptly displayed may be accounted for by three well-known fermentative changes: (1) The destruction of the free acids of the green manures, probably by molds; (2) the destruction of a part or all of the combined organic acids, liberating the alkaline substances with which they were combined in the fresh plant material, so that these alkaline materials can aid in turn to satisfy part of the original requirement of the soil; (3) the conversion of the nitrogenous material of the manures to ammonia, which can, like the bases of the organic salts, serve as alkali for the soil.

No analytical studies suited to determine whether any or all of these causes had actually operated in these experiments were made. We have, however, some indirect evidence upon the subject.

In the first place, the surfaces of the mixtures were promptly covered with thick felts of white mold. The presence of organisms capable of rapid destruction of the organic acids of the green manures is therefore established.

In the second place, the degree of the change in limestone requirement and the neutralizing effect possible from the complete ammonification of all the nitrogenous substances of the manures, and that possible from the complete liberation of all the alkaline ash constituents of the manures, may exhibit relations serviceable for this study. These facts are set forth in Table V, the ammonia and alkaline ash figures being expressed in the calcium-carbonate equivalent for 20 tons of dry matter of the respective manures, the proportion mixed with the soil in these experiments.

TABLE V.—*Alkalinity and ammonia production in soils by green manures*

[Results expressed as the calcium-carbonate equivalent for 20 tons of dry manure]

Material.	Decrease in limestone requirement.	Ammonia equivalent.	Ash alkali equivalent.
	Pounds.	*Pounds.*	*Pounds.*
Control soil	0	.....	.....
Legume mixtures:			
Soybean	1,593	3,970	2,768
Canada field pea	2,543	5,212	3,120
Sweet clover	2,503	4,455	2,108
Alfalfa	2,473	3,941	3,128
Red clover	3,950	2,770	2,252
Hairy vetch	2,420	4,056	1,308
Subaverage	2,580	4,067	2,447
Nonlegume mixtures:			
Wheat	1,135	5,069	2,400
Rape	1,377	6,840	4,192
Oats	4,244	4,398	2,140
Corn	2,942	3,884	1,880
Rye	2,503	1,899	508
Timothy	2,033	1,842	928
Redtop	1,870	1,528	640
Sorrel	4,004	2,827	1,468
Subaverage	2,511	3,536	1,769
Barnyard-manure mixture	1,457	2,242	2,208
Grand average	2,468	3,662	2,070

In the average case, therefore, the nitrogen in the added manure would suffice to work the observed change if it were fully converted to ammonia within the brief period of two weeks. On the other hand, the ash constituents of the manure would, even though completely liberated, not suffice to work the observed change.

There are exceptions to these deductions from averages. In the cases of red clover, rye, timothy, redtop, and sorrel, the ammonia equivalent to the total nitrogen of the green manures would not suffice to effect the observed change. Indeed, in the case of the rye, the alkali equivalent of the rye, nitrogen, and ash together would not account for the entire change observed.

Moreover, there is little probability of so complete conversion of the manurial substances within two weeks as this comparison requires.

Some notion of the behavior of this acid soil with respect to the fermentative process of ammonification is afforded by the studies of Dr. G. C. Given, of the Pennsylvania Station. He found that the soil of plot 32 (the source of the soil used in the present experiment), first sterilized by heat and then inoculated by the bacteria from neighboring fer-

tile soil, changed to ammonia in seven days from one-third to one-half of the nitrogen added as dried blood or as cottonseed meal. In an earlier experiment the same investigator found that the bacteria from the soil of plot 32, when used to inoculate another previously sterilized soil, acted with like ammonifying vigor as those of the first mentioned of these two experiments.

Without attempting to apply these proportions to the conditions of our own experiments we may at least infer that during the first two weeks a very advanced stage of ammonification might be reached in the case of the highly fermentable fresh green manures.

INCREASE OF LIME REQUIREMENT AT LATER PERIODS

The facts presented in Tables III and IV show, as already has been pointed out, several general increases of limestone requirement in the manurial mixtures.

It is well known that among the oxidation processes of general occurrences in ordinary plowed land is that bacterial fermentation which results in the transformation of ammonia to nitric acid. The effect of such a change is clearly to increase the limestone requirement, because the nitric acid must combine with basic materials to form neutral salts. The degree of effect varies, however, for each unit quantity of nitric acid produced, according to the conditions in which the requisite nitrogen exists at the beginning of the period of observation. If, on the one hand, it is present in a body that has little or no effect on the reaction of the soil medium, then the conversion to nitric acid will change the acidity only in such measure as the nitric acid itself contributes to the acidity; on the other hand, if the nitrogen is already present as ammonia, it serves in the measure of its quantity to reduce the limestone requirement at that time, but upon conversion to nitric acid it not only adds to the acid but subtracts in like measure from the previous series of the alkaline substances, so that, in this case, it works a double effect. In the former of these alternatives 28 pounds of nitrogen would make enough nitric acid to require 100 pounds of carbonate of lime for its neutralization; in the latter case the same quantity of nitrogen in the form of ammonia would perform the neutralizing service of 100 pounds of carbonate of lime, but upon conversion to nitric acid would not only lose its former power but would require the direct action of an equal quantity of the calcium carbonate, so that the net effects of the conversion of the 28 pounds of ammoniacal nitrogen to nitric nitrogen are to increase the calcium carbonate duty of 200 pounds. There are several other alternative conditions possible. In one ammonia might be formed from a neutral body at the same rate as the nitric acid itself is generated. Of course, in this case the one substance exactly balances the other in its effect upon the limestone requirement.

Tables VI and VII give the quantities of nitric nitrogen present in our experimental materials at the second period of observation and the changes in these quantities during the later intervals. The determinations began with the 4-week period.

TABLE VI.—*Nitric nitrogen in manured soils (Series I)*

[Results expressed as pounds of nitric nitrogen per 2,000,000 pounds of soil]

Material.	Period 2 (4 weeks).	Period 3 (3 months).	Period 4 (5 months).	Period 5 (7 months).	Period 6 (9 months).	Average through last 8 months.
Control soil	26. 54	40. 84	40. 88	57. 50	62. 70	45. 68
Legume mixtures:						
Soybean	46. 00	70. 84	136. 24	136. 98	143. 06	106. 62
Canada field pea	51. 10	115. 92	127. 02	115. 00	139. 98	109. 80
Sweet clover	21. 52	96. 02	115. 00	169. 46	157. 04	111. 80
Alfalfa	26. 48	111. 08	131. 42	161. 00	169. 46	119. 88
Red clover	3. 58	33. 94	184. 00	184. 00	246. 00	130. 62
Hairy vetch	39. 52	58. 88	105. 10	128. 80	140. 32	94. 52
Subaverage	31. 37	81. 11	133. 13	149. 21	149. 31	112. 21
Nonlegume mixtures:						
Wheat	76. 36	138. 00	147. 20	238. 50	207. 46	161. 50
Rape	60. 70	128. 72	216. 48	214. 64	207. 48	165. 80
Oats	21. 52	81. 74	118. 68	134. 18	153. 18	101. 86
Corn	43 04	98. 12	115. 12	153. 32	161. 00	114. 12
Rye	14. 36	44. 16	58. 88	80. 50	86. 98	56. 96
Timothy	2. 88	5. 74	27. 54	52. 76	64. 40	30. 66
Redtop	7. 64	11. 96	42. 92	53. 64	68. 48	36. 92
Sorrel	1. 76	15. 04	48. 44	59. 62	80. 50	41. 06
Subaverage	28. 53	65. 56	96. 91	123. 64	133. 68	88. 61
Barnyard manure mixture	51. 06	53. 82	79. 54	84. 74	107. 32	75. 28
Poultry manure mixture[a]	(b)	176. 64	245. 32	214. 64	257. 60	223. 40
Grand average	31. 17	71. 00	110. 24	131. 28	138. 18	97. 16

[a] Omitted from grand average. [b] Lost.

By reference to the grand average of Table IV, it is seen that, in general, the second 2-week period and that covering the fourth and fifth months were marked by great increases in the lime requirement of most of the manurial mixtures.

The data for nitric nitrogen exhibit no corresponding increase in the formation of nitrates, or at least in their accumulation, even if we assume that all found at the end of the fourth week were formed in the last 14 days of that time, which is not probable.

TABLE VII.—*Periodic changes in nitric nitrogen (Series I)*

[Results expressed as pounds of nitric nitrogen per 2,000,000 pounds of soil]

Material.	Second and third months.	Fourth and fifth months.	Sixth and seventh months.	Eighth and ninth months.
Control soil	14.28	0.06	16.62	5.20
Legume mixtures:				
Soybean	24.84	65.40	.74	6.08
Canada field pea	64.82	11.10	−12.02	24.98
Sweet clover	74.50	18.98	54.46	−12.42
Alfalfa	84.60	20.34	29.58	8.46
Red clover	30.36	150.06	0.00	62.00
Hairy vetch	19.36	46.22	23.70	11.52
Subaverage	49.75	52.02	16.08	16.77
Nonlegume mixtures:				
Wheat	61.64	9.20	91.30	−31.04
Rape	69.02	86.76	−1.84	−7.16
Oats	60.22	36.74	15.50	19.00
Corn	55.08	17.00	38.20	7.68
Timothy	2.86	21.80	25.22	11.64
Rye	29.80	14.72	21.62	6.48
Redtop	4.32	30.96	10.72	14.84
Sorrel	13.28	33.40	11.18	20.88
Subaverage	37.02	31.32	26.49	5.29
Barnyard-manure mixture	2.76	25.72	5.50	22.58
Poultry-manure mixture [a]	(?)	68.68	−30.68	42.96
Grand average	39.83	39.23	20.90	11.03

[a] Omitted from grand average.

In the second place, even under the assumption according to which the nitrification would most largely increase the limestone requirement—namely, that the nitrates were formed from the previous stock of ammonia, and that the ammonification was entirely completed prior to the period in question—the quantities of nitric nitrogen found are altogether insufficient to account for the large development of the limestone requirement. Thus, if the 31.17 pounds of nitric nitrogen found at the end of the fourth week are assumed to have formed during the preceding 14 days, and it is further assumed that ammonification was arrested during that time, the corresponding increase of the average limestone requirement would be only 222.5 pounds, as compared with the observed increase of 1,261 pounds; and, in this way, the 39.23 pounds increase of nitric nitrogen during the fourth and fifth months could account for no more than 280 pounds of the 1,596 pounds increase in limestone requirement observed for that period.

The further fact that the second and third month witnessed a decrease in the limestone requirement and that this condition prevailed also, in the average case, during the eighth and ninth months, whereas nitric nitrogen

increases occurred during these two periods, is also illustrative of the lack of correspondence between the degrees of soil acidity, or limestone requirement, and of nitrification in these experiments.

While, therefore, nitric nitrogen is contributory to the effect in all soil acidity, it is by no means the chief factor producing that effect in these cases.

In passing, we may note several other points upon which these experiments throw some light. The average legume yielded in nine months more nitrates than the average nonlegume green manure, and both gave more than stable manure, but less than poultry manure. At the top of the list, exclusive of poultry manure, are red clover, wheat, and rape; at the bottom, soybeans, timothy, and redtop. Among the nonlegumes, wheat and rape held the most nitrogen and had potentially the most alkaline mineral matter, but red clover had, among the legumes, the least nitrogen and by no means the most alkaline ash. These properties were therefore not the factors that determined the respective rates and degrees of nitrification.

On comparing the manurial mixtures with the control soil, it appears that at the end of the first month, there was, on the average, little difference between the manured and unmanured soils; but the departures from the average are relatively numerous. The red-clover mixture had less nitrates than the control soil until after the third month, but greatly exceeded it thereafter; and the timothy, redtop, and sorrel mixture did not catch up to the untreated soil for about five months, and thereafter differed little from it; in all other cases than the last three named, however, the manured soils surpassed the control soil in nitrate accumulation.

These cases are simply cumulative with the other laboratory and field studies previously made upon these quite highly acid or lime-requiring soils from plot 32, as to the fact that their acid properties do not prevent a fairly vigorous nitrification. With respect to this point, the accumulation of nitrates in the wheat mixture during the last four months of the experiment, when its limestone requirement was upward of 7,000 pounds to the acre 7 inches is to be especially noted.

SERIES II: AIR-DRIED GREEN MANURES

For Series II the same leguminous plant material except Canada field peas was used as in Series I, but mixed air-dry instead of fresh with the soil; of the nonlegumes only five materials were used in the air-dry state—namely, rape, corn, rye, redtop, and sorrel. Aside from the air drying of the green manures, the experiments of Series II were like those of Series I, except that the limestone requirements and nitric nitrogen were not determined at the 4-week and 5-month periods.

The results of the several determinations made are given in Tables VIII to XI.

TABLE VIII.—*Limestone requirement of air-dried green manures (Series II)*

[Results expressed as pounds of limestone per 2,000,000 pounds of soil]

Material.	Period 1 (2 weeks).	Period 3 (3 months.)	Period 5 (7 months).	Period 6 (9 months).	Average for 9 months.
Control soil	4,644	4,644	4,871	4,904	4,765
Legume mixtures:					
Soybean	3,860	4,169	5,568	5,722	4,817
Sweet clover	3,325	5,515	5,345	6,536	5,179
Alfalfa	3,965	4,939	5,481	5,790	5,043
Red clover	1,763	2,237	4,203	3,626	2,957
Hairy vetch	4,847	5,277	6,366	6,265	5,688
Subaverage	4,400	4,427	5,393	5,588	4,737
Nonlegume mixtures:					
Rape	4,871	5,790	5,616	5,515	5,448
Corn	3,321	5,210	6,910	6,944	5,596
Rye	3,592	3,897	5,616	5,960	4,766
Redtop	3,288	4,304	5,176	5,855	4,656
Sorrel	3,187	3,085	4,813	5,481	4,141
Subaverage	3,652	4,457	5,626	5,951	4,921
Grand average	4,066	4,442	5,509	5,769	4,829

TABLE IX.—*Changes in the limestone requirement of soils (Series II)*

[Results expressed as pounds of limestone per 2,000,000 pounds of soil]

Material.	First two weeks.	From end of second week to end of third month.	From end of third to end of seventh month.	Eighth and ninth months.
Control soil	0	0	227	33
Legume mixtures:				
Soybean	−403	−957	309	154
Sweet clover	−511	−1,858	2,194	1,191
Alfalfa	−345	−1,078	974	309
Red clover	−1,706	−1,194	474	−577
Hairy vetch	386	−841	430	−101
Subaverage	−516	−1,186	876	195
Nonlegume mixtures:				
Rape	−234	−577	919	−101
Corn	−1,278	−2,275	1,889	−34
Rye	−991	−1,174	305	344
Redtop	−62	−1,368	1,016	679
Sorrel	−1,592	−954	−102	668
Subaverage	−831	−1,270	805	311
Grand average	−674	−1,228	841	253

TABLE X.—*Nitric nitrogen in air-dried green-manured soils*

[Results expressed as pounds of nitric nitrogen per 2,000,000 pounds of soil]

Material.	Period 3 (3 months).	Period 5 (7 months).	Period 6 (9 months).	Average for 9 months.
Control soil	40. 84	57. 50	62. 70	53. 68
Legume mixtures:				
Soybean	98. 12	128. 80	161. 00	129. 30
Sweet clover	53. 82	107. 18	123. 82	94. 94
Alfalfa	98. 90	129. 08	169. 46	132. 48
Red clover	52. 54	86. 98	107. 32	82. 28
Hairy vetch	58. 88	107. 18	128. 80	98. 28
Subaverage	72. 25	111. 84	138. 08	107. 46
Nonlegume mixtures:				
Rape	118. 46	173. 88	238. 50	176. 94
Corn	55. 20	107. 18	110. 14	93. 84
Rye	35. 60	64. 40	68. 50	56. 16
Redtop	24. 52	46. 00	58. 41	42. 78
Sorrel	26. 96	78. 52	80. 50	61. 98
Subaverage	52. 15	95. 00	113. 01	86. 34
Grand average	62. 20	102. 92	117. 44	96. 90

TABLE XI.—*Changes in nitric nitrogen of air-dried green manures*

[Results expressed as pounds of nitric nitrogen per 2,000,000 pounds of soil]

Materials.	End of third to end of seventh month.	During eighth and ninth months.
Control soil	16. 66	5. 20
Legume mixtures:		
Soy bean	30. 68	32. 20
Sweet clover	53. 36	16. 64
Alfalfa	30. 18	40. 38
Red clover	34. 44	23. 34
Hairy vetch	48. 30	21. 62
Subaverage	39. 39	26. 64
Nonlegume mixtures:		
Rape	55. 42	64. 62
Corn	51. 98	11. 96
Rye	28. 80	4. 10
Redtop	21. 48	12. 41
Sorrel	51. 56	1. 98
Subaverage	41. 85	19. 01
Grand average	40. 62	23. 32

Since the same materials throughout were not used in Series I and II, the figures from the analyses of the mixtures of those materials used in

Series I, that were also used in Series II, and representing likewise the same periods as are covered by the figures of Series II, have been grouped in the following tabulations (Tables XII and XIII):

TABLE XII.—*Average limestone requirement and nitric nitrogen in manured mixtures*

[Results expressed in pounds per 2,000,000 pounds of soil]

Materials.	Limestone requirement.						Nitric nitrogen.			
	Original.	Period 1 (2 weeks).	Period 3 (3 months).	Period 5 (7 months).	Period 6 (9 months).	Average for months.[a]	Period 3 (3 months).	Period 5 (7 months).	Period 6 (9 months).	Average for months.[a]
Control soil	4,644	4,644	4,644	4,871	4,904	4,766	40.84	57.50	62.70	53.68
Fresh green manure:										
Legume	5,253	2,664	3,532	5,775	5,136	4,277	54.15	156.05	151.18	120.69
Nonlegume	5,789	3,214	3,980	5,793	5,251	4,560	59.80	112.34	120.89	97.68
Air-dry green manures:										
Legume	5,253	4,480	4,427	5,393	5,588	4,737	72.25	111.84	138.08	107.39
Nonlegume	5,789	3,652	4,457	5,626	5,951	4,921	52.15	94.00	113.01	86.39
Stable manure	(?)	3,187	3,797	5,549	4,575	4,277	53.82	84.74	107.32	81.96

[a] The averages in the last column of this table represent only the data for the third, fifth, and sixth periods.

TABLE XIII.—*Average changes in limestone requirement and nitric nitrogen*

[Results expressed in pounds per 2,000,000 pounds of soil]

Materials.	Limestone requirement.				Nitric nitrogen.	
	First 2 weeks.	End of second week to end of third month.	End of third to end of seventh month.	Eighth and ninth months.	End of third to end of seventh month.	Eighth and ninth months.
Control soil	0	0	237	33	16.66	5.20
Fresh green manures:						
Legume	−2,589	868	2,243	−941	101.90	−4.87
Nonlegume	−2,575	766	1,813	−691	52.54	8.55
Air-dry green manures:						
Legume	−773	−53	1,966	−851	39.59	26.24
Nonlegume	−2,137	805	1,169	−1,031	41.85	19.01
Stable manure	(?)	610	1,752	−974	30.92	22.58

It is evident from Table XII that the fresh green manures affected the limestone requirement in greater degree and for a longer period than the corresponding air-dried green manures; that the changes in lime requirement after the first period of fermentation were not so great in case of the air-dried as in that of the fresh material; and that, with a single exception, this requirement did not reach so high a point with the air-dried materials as with the fresh, the exception noted being for the nonlegume materials in the last months of the experiment. The limestone requirement of the air-dried mixtures, as contrasted with that of the soil untreated, was in general about on a level with the latter until the end of the third month, after which it became considerably higher.

As to nitrates, the accumulation did not reach so high a point during the time of the experiment with the air-dried manures as with the fresh.

In general, the evidence indicates that none of the major fermentations here concerned progressed as rapidly with the air-dried as with the fresh materials. Wollny[1] has observed a like decrease in the rapidity of fermentation with fresh plants air-dried and remoistened.

ORGANIC MATTER OF MANURED SOILS

The quality of sourness, or acidity, is sometimes traceable to the condition of the mineral constituents of soils sometimes to that of their organic constituents. In the case of the soil used for these experiments, the factors conditioning its acid or lime-requiring qualities have not been at all completely worked out.

It is, however, a silty soil rather than one rich in clayey constituents, and on the fertilizer plots, other than those treated with ammonium sulphate, has not exhibited a high-degree lime requirement, though, elsewhere in the vicinity, when under grass for a long time or even long farmed by the common rotation, it gradually acquires acid properties.

In the case of the present experiment, however, the differences exhibited as the result of the addition of manures chiefly composed of organic substances must be due, either directly to the fermentation residues of these manures or indirectly to changes they induce in the soil constituents, organic or inorganic.

To gain some idea of some of the relationships of the organic residues from the green manures, the several mixtures were each thoroughly remixed, passed through a sieve of 40 meshes to the inch, and submitted to additional analytical examination.

ORGANIC MATTER

The amount of the organic matter in the soils was approximated by ascertaining the loss in weight which they exhibited upon burning, correction being made for the portion of this loss that is due to the hygroscopic moisture of the respective soils.

It is recognized that the method of determination here used gives too high direct results, owing to the fact that the water of hydration of the mineral matters, the carbon dioxid of the carbonate, part of the sulphur of the sulphids, and part of any chlorin present probably escape with the gases formed from the burning of organic matter. We are here chiefly interested, however, in differences in mixtures resulting from the use of a single soil as the chief constituent, of which mixture it constituted slightly over 98 per cent of the dry matter. These differences are not, it is believed, considerably affected by the sources of error just named.

[1] WOLLNY, Ewald. DIE ZERSETZUNGEN DER ORGANISCHEN STOFFE UND DIE HUMUSBILDUNGEN. p. 115. Heidelberg, 1917.

In Table XIV are stated (1) the corrected loss of the several soils in burning, (2) the excess of such loss shown by the manurial mixture as compared with the unmanured soil, (3) the corresponding weights of these excesses in 2,000,000 pounds of the soil, (4) the weights of organic matter originally added in the manurial substances, (5) the weights, and (6) the percentages of the added organic matter that had disappeared during the nine months of the experiment.

TABLE XIV.—*Total organic matter of the manured soils (Series I)*

Material.	Losses on burning.	Excess of losses by manured v. control soil.	Weight of excess in 2,000,000 pounds.	Weight of manurial organic matter originally added.	Loss of added organic matter by fermentation.	
					Weight.	Per cent.
	Per cent.	*Per cent.*	*Pounds.*	*Pounds.*	*Pounds.*	
Control soil	4.873	.....	.....	.....	.....	.....
Legume mixtures:						
Soybean	5.304	0.426	8,520	29,216	20,696	70.84
Canada field pea	5.479	.669	13,380	35,540	22,160	62.35
Sweet clover	5.482	.604	12,080	36,864	24,784	67.23
Alfalfa	5.346	.468	9,360	36,548	27,188	74.39
Red clover	6.112	1.234	24,680	37,100	12,420	33.48
Hairy vetch	5.535	.657	13,140	37,216	24,076	64.69
Subaverage	5.543	.676	13,527	35,414	21,887	61.80
Nonlegume mixtures:						
Wheat	5.547	.669	13,380	35,744	22,364	62.57
Rape	5.315	.437	8,740	32,132	23,392	72.80
Oats	5.539	.661	13,220	35,384	22,164	62.64
Corn	5.419	.541	10,820	36,516	25,696	70.37
Rye	5.812	.939	18,780	38,160	19,380	50.81
Timothy	5.746	.868	17,360	37,504	20,144	53.71
Redtop	5,644	.766	15,320	37,596	22,276	59.25
Sorrel	5.455	.577	11,540	36,072	24,532	68.01
Subaverage	5.560	.682	13,645	36,138	22,483	62.21
Barnyard manure mixture	5.563	.685	13,700	24,352	10,652	43.74
Poultry manure mixture [a]	5.368	.490	9,800	28,552	18,752	65.68
Grand average	5.553	.680	13,601	35,063	21,462	61.21

[a] Omitted from grand average.

With respect to the soils of Series I, these general facts appear: All the organic manures left the soil richer in organic matter nine months after their addition. In the application of this result to practice it must be recalled that the moisture condition was most favorable and that the temperatures were also somewhat more favorable to fermentation and decomposition than a corresponding nine months outdoor season from March to November would be. Furthermore, it is to be recalled that the soil was a fine silty loam, and was, on the one hand, less favorable

to decomposition than a loose sandy soil would be, and more favorable, on the other, than would be the case with a compact clay soil.

TABLE XV.—*Total organic matter of the green-manured soils (Series II)*

Material.	Losses on burning.	Excess of losses by manures v. control soil.	Weight of excess in 2,000,000 pounds.	Loss of added organic matter by fermentation.	
				Weight.	Per cent.
	Per cent.	*Per cent.*	*Pounds.*	*Pounds.*	
Control soil	4.878	.....	.....	.....	.....
Legume mixtures:					
Soybean	5.465	0.587	11,740	17,476	59.82
Sweet clover	5.541	.663	13,360	23,504	63.33
Alfalfa	5.586	.708	14,160	22,388	61.25
Red clover	5.740	.826	16,520	20,580	55.47
Hairy vetch	5.720	.843	16,860	20,356	54.70
Subaverage	5.611	.733	14,660	20,861	58.73
Nonlegume mixtures:					
Rape	5.408	.530	10,600	21,532	67.01
Corn [a]	.....	.....	.....	.....	.....
Rye	5.620	.742	14,840	23,320	61.11
Redtop	5.723	.854	17,080	20,516	54.57
Sorrel	5.698	.821	16,420	19,652	54.48
Subaverage	5.612	.734	14,680	21,255	59.15
Grand average	5.612	.734	14,670	21,036	58.92

[a] Sample lost.

There was no appreciable difference in the proportion of loss of the organic matter of the groups of legumes and nonlegumes, respectively, and both suffered greater loss than stable manure, which had doubtless been considerably changed by fermentation before application.

There was, however, a good deal of difference in the degree of loss exhibited by individual materials. The figure for red clover is surprisingly low, and those for rye and timothy are much below the average.

We may question whether there is any direct relation between the amount of total residual humus and the limestone requirement at the end of the nine months period. We are at once confronted by the fact that where alfalfa, red clover, hairy vetch, rye, timothy, redtop, and sorrel were added to the soil, its limestone requirement was less than that of the untreated soil, and yet in each of these cases there was a large residue in the soil of organic matter from the green manures. We may, therefore, not regard the organic residues as active, as a whole, to cause the observed acidity. When we consider the variety of organic matters originally present, and the differences in the mutual proportions they originally bore to one another, it would at once appear highly improbable that the residues from their fermentation would have like acid effects for equal units by weights.

Considering the fresh and air-dried green-manure mixtures as two groups, we find, if we include in these groups only those materials represented in both series, these differences:

	Applied fresh.	Applied air-dry.
Excess of organic matter over soil:		
Per cent	0.679	0.734
Pounds in 2,000,000	73,580	74,680
Loss by soil fermentation:		
Pounds in 2,000,000	22,083	20,976
Per cent	61.91	58.63

That is, at the end of nine months there was lost by fermentation about 1,100 pounds more of the organic matter applied in a fresh green state than in an air-dried green state. This amounts to but 3.3 per cent of the total application of organic matter. The delayed fermentation of the air-dried material, the rather marked effect of which fermentation difference with respect to both limestone requirement and nitrate development has received earlier comment, resulted finally in much less difference in total decomposition than might have been forecast from the soil acidity and nitrification differences.

Several years ago in studying the grass roadways bounding the several tiers of the General Fertilizer Series of plots, it was found that these roadways differed much in their lime requirement, and that in each instance the lime requirement bore a fixed ratio to the amount of the free humic acid—that is, that portion of the humus that was soluble directly in weak alkali without the previous removal of lime, etc., by acid washing. The constant ratio observed in that grass-land soil was 11.27 of the free humic acid to 1 of lime.

To determine whether these green-manuring soils would exhibit a like condition with the grass lands mentioned above and also more generally to ascertain whether the humus of the several green-manuring soils, as they existed nine months after the application of the manures, was possessed of peculiarity in the proportion of alkali-soluble humus—sometimes called "active humus"—this group of constituents was determined by a modification of the McBride method in which the filtration of the alkaline liquid was performed by the aid of Chamberland-Pasteur filters, which effectually remove suspended matters and yield a clear filtrate. The weakly alkaline solution was made to act upon the soil in two conditions:

A.—Ten gm. freed from basic materials by washing with 1 per cent of hydrochloric acid and then with water was shaken for 15 hours in a Wagner shaking apparatus with 1,000 c. c. of 4 per cent ammonia water, the suspended matter allowed to settle, the overlying liquor then filtered in the manner just stated, and an aliquot of the filtrate evaporated in a weighed platinum dish and dried at 103° C. for four hours. The material

was then burned in this dish, the loss in weight counted as free and combined humic acid, and the mineral residue as humus ash.

B.—An equal weight of the soil was treated in exactly the same way, except that the removal of the bases by washing with hydrochloric acid and water was omitted. The loss on burning the alkali-soluble material obtained in this manner was counted as free humic acid and the associated humus ash determined as in the preceding case.

It is to be observed that if any portion of the organic matter is soluble in either weak hydrochloric acid or water, or both, and also in weak ammonia water, a loss of this fraction of the material would occur with the treatment by the usual method, A, but not by method B. In such case these two treatments would not give the full measure of the material, free and combined with basic matter, that is capable of solution in the weak alkali.

TABLE XVI.—*Determinations of humus and humus ash in experiment mixtures (Series I and II)*

Material.	Fresh manures.				Air-dry manures.			
	Humus ash.		Soluble humus.		Humus ash.		Soluble humus.	
	A	B	A	B	A	B	A	B
	Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
Control soil	0.48	0.57	1.23	1.48	.....	.....	.....	.....
Legume mixtures:								
Soybean	.....	.....	1.30	1.80	0.45	0.40	1.39	1.64
Canada field pea	.....	.....	1.35	1.65	.....	.....	.....	.....
Sweet clover	.....	.....	1.32	1.66	.41	.44	1.41	1.65
Alfalfa	.....	.....	1.59	1.70	.40	.47	1.40	1.69
Red clover	.....	.....	1.61	1.88	.43	.94	1.38	1.67
Hairy vetch	.....	.....	1.40	1.63	.35	.45	1.44	1.74
Subaverage	.....	.....	1.43	1.72	.41	.54	1.40	1.68
Nonlegume mixtures:								
Wheat	.....	.....	1.36	1.81	.....	.....	.....	.....
Rape	.....	.....	1.25	1.61	.38	.53	1.33	1.59
Oats	.....	.....	1.39	1.75	.....	.....	.....	.....
Corn	.....	.....	1.35	1.63	.....	.....	.....	.....
Rye	.....	.....	1.41	1.78	.49	.47	1.41	1.65
Timothy	.....	.....	1.48	1.66	.....	.....	.....	.....
Redtop	.....	.....	1.38	1.61	.33	.51	1.50	1.73
Sorrel	.....	.....	1.38	1.65	.36	.53	1.43	1.66
Subaverage	.....	.....	1.37	1.69	.39	.51	1.44	1.61
Barnyard-manure mixture	.....	.....	1.33	1.70	.....	.....	.....	.....
Poultry-manure mixture [a]	.....	.....	1.27	1.55	.....	.....	.....	.....
Grand average	.....	.....	1.39	1.70	.40	.53	1.42	1.67
Fresh humus, subaverage:								
Legume	.....	.....	.....	.....	.....	.....	1.44	1.73
Nonlegume	.....	.....	.....	.....	.....	.....	1.35	1.66

[a] Omitted from grand average.

The comparison of the results obtained by the two methods of determining the alkali-soluble humus in this acid soil, and in its fermented mixtures with various organic manurial substances, shows strikingly that more organic material was obtained by the direct solvent action of ammonia (method B) than by washing with weak hydrochloric acid and water for the purpose of breaking up the humate combinations with basic substances and then treating with the dilute ammonia water (method A). The mineral matters associated with the dissolved humus are more abundant with the former treatment than with the latter; but even in the two individual cases where the reverse is true, the direct action of the ammonia removed more organic material.

From these facts we may conclude that in this acid soil, both untreated and as modified by fermentations of organic manures applied, very little of the "active humus" is combined with bases; that washing with weak acid and water removes a considerable amount of the organic matter and usually some basic materials.

It is further clear that the data obtained by these analytical methods are not such as to make it possible to establish a relation between the limestone requirements of the respective soils and a particular fraction of their active humus.

The facts set forth in Table XVI do not show any noteworthy difference between the soil percentages of active humus in the mixtures obtained from fresh as compared with the air-dried manures.

We may present, however, a comparison from the data of Tables XV and XVI to show the respective proportions in which the total humus in the several groups of manurial mixtures, in excess of that accounted for by the untreated soil, is composed of active humus as determined by the usual method A. The proportion shown by the soil itself is added for further comparison (Table XVII).

TABLE XVII.—*Percentage of active humus in the organic matter of the manurial mixtures*

Material.	Total organic matter (pounds in 2,000,000).	Active humus (pounds in 2,000,000).	Percentage of active humus in organic matter.
Untreated soil	97,560	24,600	25.19
Excesses contributed by—			
Legume green manures:			
Fresh	13,556	4,000	29.36
Air-dry	14,660	3,400	20.46
Nonlegume green manures:			
Fresh	13,595	4,800	35.31
Air-dry	14,680	4,200	28.61
Stable manure	13,700	2,000	14.60
Poultry manure	9,800	800	8.16

In case of both legume and nonlegume green manures, therefore, the organic residue from the material applied fresh contains a larger proportion of alkali-soluble humus than does that from the air-dried manure. The percentage of alkali-soluble humus in the organic residue from the fermentation of stable manure in the soil is much less and that from the poultry manure is even lower.

Finally, we may consider whether these examinations exhibit any definite relation between the limestone requirement due to the added green manure and the alkali-soluble organic residues from these additions. For such purpose the results from the determination of alkali-soluble humus by method B are possibly best suited.

It will not be needful to make an extensive comparison in order to discover that no definite relation of the kind stated is here apparent. Table II shows 8 cases out of 15 in which the limestone requirements of the manured soils was less than that of the untreated soil at the end of the 9-month period; Table VIII shows one such case out of 10. On the other hand, Table XVI shows no case in which the alkali-soluble humus determined by method B in the manured soils is not greater than that of the untreated soil.

Therefore, if the chief acid effect in these cases is due to the alkali-soluble humus constituents as a whole, the influence of a unit weight of these parts of the several organic residues must be very different in degree in the respective cases.

SUMMARY

Under conditions corresponding for nine months to those of a summer fallow with fairly frequent, thorough cultivation and well-distributed rainfall of amount just sufficient to keep the soil well moist without setting the drains aflow, the following facts were observed with respect to the acid soil of the ammonium-sulphate plot 32, both as fallowed by itself, with stable manure and with various leguminous and nonleguminous manuring crops, applied fresh and also in an air-dry condition, respectively.

I.—THE SOIL WITHOUT MANURIAL ADDITION

(1) Its limestone requirements changed little, if at all.

(2) Its nitrate content increased sharply at two periods, first, during the second and third months, and at a less rate after the fifth month. If these increases represented a change from ammonia to nitric acid, without a simultaneous replacement of the transformed ammonia from organic nitrogenous sources, they would be equivalent to increases in the limestone requirement of 104 and 120 pounds, respectively, to the acre 7 inches (2,000,000 pounds).

(3) At the conclusion of the experiment the amount of nitric nitrogen was 36.16 pounds greater than at the beginning, or somewhat more than double.

II.—THE SOIL WITH STABLE MANURE ADDED

(4) The original limestone requirement of this mixture was not determined, nor were the data needful for its computation secured. There was, however, an almost continuous increase in this requirement from the end of the second week to the conclusion of the seventh month. During the third and fourth weeks the increase was 678 pounds (in 2,000,000), and during the fourth to the seventh month, inclusive, 1,652 pounds; but the two following months witnessed a decrease of nearly 1,000 pounds.

(5) The nitrates, on the other hand, increased continuously after the fourth week. This increase was greatest during the fourth and fifth months and again during the eighth and ninth months; at the one time the limestone requirement was increasing, and in the other markedly decreasing. At the utmost of its effect the nitrification can account for but a small fraction of these limestone-requirement changes.

(6) Until the end of the third month the limestone requirement of the stable manure mixture was less than that of the soil; from the fifth to the seventh month it was 1,000 pounds greater than that of the soil alone; but at the end of the experiment the soil had the greater lime requirement.

(7) At all times the stable manure mixture showed more nitrates than the soil alone.

III.—THE SOIL WITH ADDED FRESH GREEN MANURES

(8) At the end of the first two weeks the limestone requirement of these mixtures was, on the avearge, about 1,400 pounds less (in 2,000,000) than that of the soil alone. During these two weeks this requirement decreased from 5,858 pounds, the theoretical original acidity of the mixtures, to 3,044 pounds, a decrease of 2,540 pounds, or nearly one-half. As noted earlier in this summary, the soil alone showed no change in this requirement during this period.

(9) The plan of the experiment was not sufficiently complete to afford a proof of the cause of this decreased limestone requirement. Three factors may have operated to cause it: A destruction of the free acids of the green manures; a destruction also of the plant acids combined as salts with a consequent liberation of alkaline ash constituents; and the conversion of the organic nitrogen to ammonia. The first factor is too small to account for a large fraction of the observed decrease. Potentially, either of the others would suffice to account for it in most of the cases, though not in all. Complete destruction of the acid combinations in the plants within two weeks, or complete ammonification of the organic nitrogen within two weeks, seems unlikely. It is probable, therefore, that a large development of the three factors at the same time is the real explanation of the observed change.

An increase of limestone requirement in the case of these mixtures occurred during the third and fourth weeks, and again during the fifth

to sixth months, followed in the average case by but a small decrease during the eighth and ninth months. In 7 out of the 14 mixtures with fresh green manures a slight depression of the limestone requirement appeared at the end of the third month, and in but one of the remaining cases was there an important increase during this time.

(10) In the average case, though with a number of exceptions, the soil alone had a greater limestone requirement than its fresh green manure mixture up to the end of the third month, but from the end of the fifth month until the end of the experiment these mixtures on the average had the higher requirement.

(11) The course of the limestone requirement varies, however, quite differently in the several individual cases. With red clover it dropped to nothing by the end of the third month, and then jumped within the next two months to nearly 5,000 pounds, the greatest and most abrupt change appearing in these records. The highest individual limestone requirement was that of wheat at the end of the fifth month—namely, 7,594 pounds. The sorrel mixture was the only one that did not at some time exceed the soil in its limestone requirement.

(12) Among the individual green manures the highest limestone requirements on the average throughout the experiment were those of corn among the nonlegumes and of sweet clover among the legumes; the lowest, those of sorrel and red clover in these respective subgroups.

(13) In the average case these mixtures held at the end of the second week about as much nitric nitrogen as the soil; but thereafter the increase was continuous to the end of the experiment, when these mixtures held more than twice as much of this constitutent as the soil alone. In individual mixtures there were fluctuations in the quantity rather than a steady increase.

(14) The averages throughout the season show that the rape, wheat, and red clover mixtures had the most nitrates, while the timothy, redtop, and sorrel mixtures had less than the soil alone.

(15) In all these cases, as in that of the stable manure mixture, nitrification alone was absolutely insufficient to account for the observed developments of the limestone requirement.

(16) Although the green manured soil had an average limestone requirement much greater than that of the untreated soil, there was a quite strong nitrification in the presence of the green manures. In other words, here, as in the field, the acidity developed has not prevented fairly vigorous nitrate formation.

IV.—THE SOIL WITH ADDED AIR-DRY GREEN MANURES

(17) All the fermentative changes followed the same direction displayed by the mixtures with fresh green manures. The depression of the limestone requirement in the early weeks was less pronounced, the gain steadier but more delayed thereafter. The nitrification also progressed more slowly, and apparently had not reached its highest accumulation of

products at the time when the experiment ended. Under these conditions the red clover did not develop at any time a neutral soil; and at the end of the experiment all the mixtures, except that with red clover, had a greater limestone requirement than the soils alone exhibited.

V.—EFFECTS OF THE ORGANIC MANURES UPON THE AMOUNT AND CONDITION OF THE HUMUS SUPPLY OF THE SOIL

(18) The organic matter of the soil is the net result of manured and crop-root additions and the fermentative decomposition subtraction. With open soil, good moisture supply, summer temperature, and vigorous bacterial life, the additions may be matched by a greater subtraction, with no permanent gain in humus as the result. In the case of this silty loam, with such lime deficiency as is usually accompanied by a lowered activity of the fermentative soil organisms, a less rapid destruction of the organic matter should be expected.

(19) In every case the soils mixed with the various organic manures showed, nine months afterward, larger quantities of organic matter than the untreated soil.

(20) The amounts of these excesses vary much with the individual case, but on the average the amounts for legumes and nonlegumes, green manures and stable manures are well within the limits of analytical error. Soybeans, rape, alfalfa, and poultry manures left the smallest residues; red clover, rye, timothy, and redtop the greatest. The figures for the red clover residue, like those for its limestone requirement, are so peculiar that, although the results have been confirmed by repeated analyses, repetition of the experiment seems needful to establish their full correctness.

(21) The absence of a determination of total organic matter in the soil at the beginning of the experiment makes impossible the calculation of the total quantity lost by the soil and its mixtures during the experiment. By comparing the amounts in the mixtures in excess of that in the unmanured soil, at the end of the experiment, with the organic matter added in the manures, the amounts and proportions of the destruction of the added organic supplies can be approximated. This comparison indicates that, in the cases of the poultry manure and of the average green manures applied in a fresh condition, three-fifths of the added organic matter was destroyed by the soil ferments during nine months, while of the organic substance of the stable manure, partly rotted when applied, little more than two-fifths was destroyed. The largest proportion of destruction was 72.8 per cent in the case of rape; the lowest—omitting the peculiar red clover results from consideration—was 50.8 per cent in the case of rye

(22) These residual organic decomposition products, each considered as a whole, have no specific effect upon lime requirement, for while all the mixtures held at the close of the experiment more organic matter

than the unmixed soil, some of these mixtures had a greater limestone requirement than the soil itself, some a less.

(23) The green manures applied air-dry were not so largely decomposed as those applied in a fresh state. The former left about 1,000 pounds an acre more of organic residues than the former.

(24) Of the total organic residue in the untreated soil, only 25 per cent were left as "active humus" or humus soluble in dilute alkali, as determined by the usual method.

(25) Of the organic residues from the fresh green manures a somewhat larger proportion, 29 and 35 per cent for the legume and nonlegume groups, respectively, were left in "active humus" condition; but of the stable and poultry manures, much less, 14.6 and 8.2 per cent.

(26) In the mixtures with air-dry green manures the proportion of "active humus" was from one-third to one-fifth less than in the corresponding mixtures with fresh green manures.

(27) Of the "active humus" in these acid soil mixtures, little, if any, exists in ammonia-insoluble combinations with lime or other bases, and probably a considerable fraction can be dissolved by weak acid and water.

(28) The properties of these variously manured soils show no more definite relationship between their limestone requirement and the "free humic acid"—that is, the humus directly soluble in weak ammonia—than exists between the total organic matter and this requirement. If the material causes of the observed requirement belong to this major fraction of the active humus, either they are of very different composition and degree of influence or else they exist in very different quantities in the several free humic acid residues, a condition very different from that observed in old grass lands upon the same body of soil.

In general, these experiments have satisfactorily shown that fresh green manures plowed under on this acid silty loam soil reduce its acidity very soon after plowing under, but finally leave a soil of increased acidity; also that nitrification goes on in them quite vigorously under suitable moisture temperature and aerative conditions and that the green manured soils are rich in nitrates, despite the soil acidity. As to the cause of the increased acidity, beyond showing that it is not largely due to nitrification and indicating that it is in some way associated with the added organic materials or their fermentative residues, the experiments furnished little definite information.[1]

[1] The writer is indebted to Dr. Wm. Freer for his valuable assistance in the interpretation of results herein discussed.

A LEAFBLIGHT OF KALMIA LATIFOLIA

By ELLA M. A. ENLOWS

Scientific Assistant, Laboratory of Plant Pathology, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

During the summer of 1914 a bed of mountain laurel (*Kalmia latifolia*) on the Department grounds became affected by a leafspot or blight. The progress of the disease in this bed was slow, but at the end of about 18 months there was not a single plant in the bed which did not show the typical brown margins and areas described below. The disease has also been found by the writer in Rock Creek Park and in the hills about Anacostia, D. C. Cross-sections of the affected leaves showed the presence of a very delicate fungus mycelium within the tissues. Cultural studies were begun and a fungus was isolated, which has been proved to be the cause of the disease.

DESCRIPTION OF THE DISEASE

The disease is characterized by a blight or dryrot involving large areas of the leaf blade or the entire leaf. Later, it extends through the petioles into the stems and may eventually kill the entire plant. Those leaves but slightly diseased show small, irregular, dark-brown spots scattered about over the blade. In fact, the disease in its early stages, especially on young, tender leaves, is visible only with a hand lens, appearing as tiny brown specks scattered about over an area of leaf surface which appears slightly lighter green than that of the surrounding healthy portion. These spots increase in size slowly, and finally become visible as tiny brown points in clusters, surrounded by a much lighter portion of green leaf tissue (Pl. 14, B). During dry, cold weather these diseased areas may remain as definitely outlined small spots for three to four weeks or longer; but if the air is moist and warm, they soon coalesce, forming the large, seal-brown to Vandyke brown [1] areas (Pl. 14, A, C), which finally involve the entire leaf blade. The incipient stage of the disease is most frequently seen at or near the tip, or along the margin of the leaf. Sometimes it spreads from the point of infection at the tip in rather an even line across the leaf blade, but it may also follow the midrib or extend along the margins of the leaf. In the latter case the margins usually curl, the inner portion of the leaf blade then appearing convex. The badly affected leaves, petioles, and stems become very dry and brittle, and such leaves drop off rather easily.

[1] The color terms mentioned throughout the text are according to Ridgway's Color Standards (RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D. C., 1912).

ISOLATION OF THE CAUSAL ORGANISM

Numerous free-hand sections of diseased leaves were made from what appeared to be the early stages of the disease—that is, the small brown spots and the adjacent portion of leaf tissue. In a few of these sections a delicate, frequently-branching mycelium was observed. Failure to find the fungus in a larger portion of the sections studied was undoubtedly due to the fact that it is not found far beyond the browned areas, the reduction of the chlorophyll beyond these areas being due possibly to toxic products of the fungus growth.

About a dozen of the diseased leaves in all stages of infection were placed without surface sterilization on moist filter paper in petri dishes, which were kept under large bell jars. Another set of dishes was arranged in the same way, except that the leaves were surface-sterilized by immersing them for one to five minutes in 1 to 1,000 mercuric-chlorid solution. The petri dishes and filter papers used were also sterilized. After a few days the leaves which had not been sterilized were covered with dense mycelial growths of a variety of fungi: Species of Alternaria, Pestalozzia, Cephalothecium, Aspergillus, etc. Numerous pycnidia were developing on a few of the leaves. Some of these were found to be the fruiting bodies of *Phyllosticta* spp. However, the majority did not appear to belong to this genus, but they were at this time too immature for examination. After about two weeks it was evident that they were not the pycnidia of *Phyllosticta* spp. Yellow spore masses in the form of cirri were extruding from them. These cirri were found to consist of spores of two kinds: An oval, hyalin, 1-celled spore with two or more definite oil drops, and a much longer, filiform, curved or hooked spore, also hyalin and 1-celled.

In the meantime those leaves which had been sterilized before being placed in the sterile moist petri dishes showed none of the abundant fungus mycelium present on the unsterilized leaves, but did show numerous pycnidia of the same type as some of those found on the latter, and they were also extruding the same kind of long, yellow cirri. These cirri were found to contain the two kinds of spores just described.

Petri-dish poured plates were made in the usual way—that is, the leaves were first rinsed in alcohol, then immersed in a 1 to 1,000 mercuric-chlorid solution. After rinsing in sterile water, small pieces of leaf tissue representing the various stages of infection were cut out and placed in the dishes, over which was poured corn-meal agar and synthetic agar. In both media there appeared after three to four days a delicate, white mycelial growth. Transfers were made to corn-meal agar, potato, etc. After about two weeks those transfers made on potato cylinders showed numerous, dark-brown fruiting bodies which were beginning to extrude cirri in the same way as the pycnidia on the leaves. Microscopic examination showed these spores to be of the same character as those found on the leaves placed in damp chambers.

From water suspensions of these spores corn-meal agar and beef-agar plates were made for the purpose of starting single-spore cultures. Under the microscope single spores were marked and, when germination had started, were transferred to corn-meal agar slants and potato cylinders. These transfers were used for the greater part of the inoculation experiments.

INOCULATION EXPERIMENTS

INOCULATIONS INTO KALMIA LATIFOLIA

The first inoculation tests were made on July 17, 1914, on twigs of *Kalmia latifolia*, which were broken off and placed in beakers and kept on a laboratory table. A suspension of the spores (from cirri extruded from pycnidia in corn-meal cultures 5 weeks old) was made in sterile water, and the leaves were sprayed on both sides with this suspension, and then placed under bell jars for three to four days. Some of the leaves which had been pricked with a sterile needle were held as controls. All of the leaves which had been pricked when inoculated showed signs of infection at the end of 11 to 15 days. The disease, however, did not progress rapidly, being confined to tiny brown spots scattered irregularly about over the leaf blade. This was undoubtedly due to the fact that the air in the laboratory was too dry for a rapid spread of the infection. The spots were typical of those observed on the original material, and cross sections showed the presence of the same delicate mycelium in the leaf tissues. Plates were poured, and pure cultures of the fungus inoculated were obtained.

On November 4, 1914, 12 plants of *K. latifolia* were inoculated by spraying on suspension of the spores from cultures 5 weeks old. Needle pricks were made in about half of the leaves inoculated. The plants were kept in the greenhouse under inoculating cages. On the same day six branches from a very large plant of *K. latifolia* were broken off, and placed in bottles standing in water, over which bell jars were placed, and kept in the laboratory after inoculation. Some of them were inoculated simply by smearing moistened spores over the leaf surfaces, but the majority were sprayed with the suspension of spores, after which needle pricks were made.

Two plants were inoculated by spraying with spores from a fungus belonging to the genus Phyllosticta, which had developed in the damp-chamber incubation. One other plant was inoculated by placing on both leaf surfaces spores of *Alternaria* sp., which had developed also in the damp chamber, and one was inoculated with spores of *Pestalozzia* sp., needle pricks having been made in both cases.

Two plants were sprayed with sterile water, pricked with sterile needles, then placed under bell jars, and kept as controls.

In this experiment the laurel plants were kept under the bell jars for 21 days, and during this time several new leaves had started; but the

disease progressed so rapidly owing to the very moist conditions that it soon reached these new leaves through the stem, when they promptly blighted. No disease appeared in the controls.

Those plants left under the cages in the greenhouse showed the first signs of the disease on November 18 in the form of numerous, small, brown spots on the upper surfaces of the leaves. On account of the moist air of the inoculating chambers, these spots soon coalesced, forming the typical brown, brittle areas (Pl. 14, A). On December 1 every plant and branch inoculated with the fungus was badly diseased; in fact, practically all of the leaves had either fallen or become brown, dry, and brittle. The stems in each case were also infected. Poured plates were made from these inoculated plants, and the fungus inoculated was isolated in pure culture from each of the inoculated plants.

The two plants inoculated with the spores of *Phyllosticta* sp. gave no positive results; nor did those inoculated with *Alternaria* sp. and *Pestalozzia* sp. The controls, both at the greenhouse and laboratory, remained healthy.

In the manner thus outlined inoculation experiments were conducted from time to time during a period of three years, inoculating in all about 50 plants of *K. latifolia* and several dozen cut branches, most of which gave positive results. One interesting fact observed in this connection was the effect of keeping the cut branches or plants under very moist conditions under bell jars. In many cases stimulation of growth resulted, and practically no injury was noticeable in any plant or branch. In one experiment (March 4, 1915) one of the control plants was allowed to remain standing in a very moist atmosphere under a bell jar from March 4 until April 14, when it showed several new leaves and appeared to be perfectly healthy. One of the leaf-inoculated plants was also left for this same length of time, when it showed on the stem the typical pycnidia from whch the yellow cirri were extruding (Pl. 15, B).

On January 7, 1916, experiments were started to determine whether stomatal infections are possible under ordinary conditions. One plant out of six inoculated on this date by smearing moistened spores on the uninjured lower surface of the leaves showed typical diseased areas. However, this one experiment is not considered of much value, as the infection may have started through some injury too small to be seen at the time of inoculation. A microscopic examination of numerous sections failed to show hyphæ penetrating the stomata. The leaves inoculated on this plant were new ones, and very delicate, and it can be readily seen that even handling them might result in minute injuries through which the hyphæ might enter.

The experiments conducted as just described seem to afford sufficient evidence as to the pathogenicity of the fungus isolated. It is a wound parasite, or at least not an exceedingly active parasite. However, once it gains entrance to the parenchyma of the plant it will kill living tissue and may involve entire branches or even the whole plant.

INOCULATIONS INTO OTHER PLANTS

The differences morphologically are not very great between the fungus here under consideration and certain species of Phomopsis which cause diseases of *Citrus* spp., eggplant, and apple. It was therefore thought advisable to carry out cross-inoculations.

Apple, orange, and lemon fruits and young, growing eggplants were inoculated with the fungus isolated from *K. latifolia*. The fruits and plants were kept under as favorable conditions for infection as possible, and the virulence of the cultures used in each experiment was tested out by inoculation into *K. latifolia*.

No infections resulted from any of the inoculations. In the experiment on apple bacterial softrots appeared, and in one case a fruit showed a spot just at the margin of one of the inoculation pricks which appeared at first somewhat like the Phomopsis-rot of apple described by Roberts (*8*, *9*). Microscopic examination revealed very large, brown hyphæ, and cultures gave no colonies of the mountain-laurel fungus.

Sufficient experiments were performed to indicate that the species of Phomopsis here described is not the same as *Phomopsis citri* Fawcett (*6*) which causes melanose and stem endrot of Citrus fruits (*4*, *7*). In addition to its inability to infect Citrus fruits, it differs morphologically from the fungus described by Fawcett (*4*), in that the pycnidia are not embedded deeply in the tissues of the host, and in no culture medium was there formation of Achyla-like branches with protrusion of the protoplasm from the ends, such as Fawcett describes.

It is not infectious to eggplant, and therefore not the same as *Phomopsis vexans* Harter (*10*), from which it differs also in pycnidial characters—for example, there is no beak either on the host or in culture media.

The negative results from the inoculations into apple distinguish it from *Phomopsis mali* Roberts (*9*), though morphologically it is very much like this species, with the exception that the spores are smaller, and often contain more than two oil drops.

EFFECT OF THE FUNGUS ON THE TISSUES

Microscopic examination of sections fixed in Carnoy's fluid and stained by Van Gieson's method shows that the diseased areas consist of a confused mass of disintegrated leaf tissue and mycelial cells. The fungus is seen to extend not far beyond these diseased areas; and this, together with the fact that the mycelium is extremely delicate and hyalin, makes it rather difficult to locate in the host tissues. Very young spots, sectioned horizontally and stained by special stains, such as Van Gieson's, give best results. In such sections the fungus may be seen ramifying through the intercellular spaces both in the palisade tissue and in the spongy mesophyll. The chloroplasts are greatly reduced in number for a considerable distance around the diseased area, and the nuclei

show signs of disintegration—that is, they lose chromatin and become more or less fragmented. The vascular portions of the leaf are not involved until such time as the toxic substances resulting from growth of the fungus have brought about disorganization of all the other tissue systems. When this occurs, a mass of disintegrated cells is seen interspersed with a few cells still retaining their original contour, but whose protoplasmic contents have been changed to dark-colored, more or less opaque coagulation products. In early stages the diseased cells may contain one to several brown, oily-appearing droplets, surrounded by either a few of the still intact chloroplasts and a fragmented nucleus, or dense coagulation products.

MODE OF ENTRANCE

From the experiments here reported it is apparent that the fungus is a wound parasite. As already noted in one experiment infection followed inoculation by spraying on a suspension of the spores in water, no needle-pricks being made. However, it was not possible in this case to demonstrate by microscopic sections that the hyphae had entered through the stomata, although a good many sections, both vertical and horizontal, were carefully examined. Slight wounds are almost always present, through which the hyphae might easily enter.

CULTURAL STUDIES

CULTURAL CHARACTERS

It was found that the fungus grows most rapidly, and produces the greatest abundance of pycnidia on steamed corn meal in flasks, and on steamed potato cylinders standing in test tubes in about 1 c. c. of distilled water. Transfers were made from these media to corn-meal agar; steamed string beans; beef agar; synthetic agar,[1] Kalmia agar; steamed leaves and twigs of *K. latifolia;* steamed corn meal plus litmus milk; litmus milk; litmus agar plus various sugars (saccharose, maltose, lactose, dextrose, galactose, mannit); plain litmus agar; corn-meal agar plus saccharose, maltose, and dextrose; Uschinsky's solution; Cohn's solution; Dunham's solution; peptonized beef bouillon; beef gelatin; milk; sterile distilled water; potato juice in fermentation tubes. On a majority of these media few or no pycnidia are formed. On steamed corn meal the spores germinate in 24 to 48 hours, forming a pure-white, delicate mycelium which soon covers the surface of the

[1] Prepared according to the formula furnished this laboratory by Mr. Frederick V. Rand: (*a*) 1,500 c. c. of distilled water and 36 gm. of agar. Cook in double boiler for one hour at 15 pounds, pressure. (*b*) 500 c. c. of distilled water, 200 gm. of dextrose, 40 gm. of peptone, 20 gm. of ammonium nitrate, 5 gm. of magnesium sulphate (crystals), 10 gm. of potassium nitrate, 5 gm. of potassium acid phosphate, and 0.2 gm. of sodium chlorid. Boil in a double boiler for 30 minutes, add agar, and cook for 5 minutes. Restore volume, titrate, cool to 60° C., and add whites of two eggs. Cook to coagulate eggs, filter, tube, and sterilize. This formula is modified from that given by Darwin and Hamilton. (DARWIN, FRANCIS, and ACTON, E. H., PRACTICAL PHYSIOLOGY OF PLANTS. ed. 3, p. 68. Cambridge, 1901.)

medium as a flat, furry layer. At the end of about 7 days a dark greenish tinge appears about the center or at the margins of the corn meal, followed by minute dark-green to black points, — the beginnings of the pycnidia. In about 15 to 20 days after inoculation the entire surface, sides, and often the bottom of the corn meal are covered with velvety, irregularly shaped, large fruiting bodies frequently having dark-green to black caps, and pure-white to light-gray and olive-green sides (Pl. 16, A, B). Sometimes when the pycnidia are very small (within 13 to 15 days after inoculation of the flask), the surface and sides of the medium will become yellow from the extrusion of the waxy coils and roundish masses of spores. At other times bodies are produced that are identical externally with the pycnidia, but which form no spores at all, even when held for months under various conditions. The spore masses vary in color, but are usually waxy yellow through amber to cream.

A greater abundance of mycelium is produced on steamed potato cylinders than on any other medium. Pycnidia frequently form within five days after inoculation, and vary in color from deep mouse-gray to dark ivy-green. When old, the seal-brown to black stroma is sharply marked with wavy white bands, and is tough and leathery in texture.

On corn-meal agar very little mycelium is produced. The pycnidia are rather small and often clustered, but form rapidly. Spores are produced promptly within the scattered groups of pycnidia, and but very few sterile bodies are formed.

Synthetic agar is a very good medium for the isolation of the fungus, as the mycelium grows quickly and abundantly, the medium at the same time inhibiting or preventing the growth of most intruding bacteria. The colonies are very beautiful, feathery in appearance, and of a color between pale dull gray and white. No pycnidia or sclerotia have ever been observed on this medium.

When litmus milk was added to steamed corn-meal flasks, the substratum was blued very decidedly at first, but at the end of 10 days was bleached almost throughout (cream), the control flasks remaining lilac.

In litmus milk a fair growth occurred, with coagulation of the milk at the end of five days, but the casein was not precipitated until the seventh day. The litmus was slightly reduced after 15 days, and at the end of 30 days was entirely reduced and the milk proteolyzed. In one tube proteolysis occurred in 13 days.

The growth of the fungus in Cohn's solution demonstrates its ability to obtain nitrogen from ammonium salts. There was no liquefaction of the medium when the fungus was grown in beef gelatin, and no pycnidia were formed.

GERMINATION TESTS

Practically all the transfers made during the work with this fungus have been germination tests, since the extruded spores were in almost every instance used to make the transfers. Spores freshly extruded germinate very promptly under favorable conditions—that is, within 24 hours they usually send out a germ tube from the blunter end, and rarely two, one from each end (fig. 1, A). Spores which are older and which have become dried of course germinate more slowly, though the age and condition of the spores and time required for germination vary in this direct ratio only up to a certain point. Long threads of spores which had stood in corn-meal flask cultures for 13 months and which had become very dry and brittle germinated about as soon as spores taken from similar cultures 6 to 8 weeks old. Steamed-potato cylinders and steamed corn meal in flasks have proved to be the best media for rapid germination.

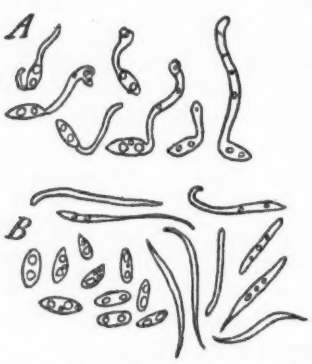

FIG. 1.—*Phomopsis kalmiae: A*, Germinating pycnospores; *B*, types of spores produced by the fungus. × 950.

Germination is very slow in distilled water, and only a small percentage of the spores introduced germinate at all. After 48 hours but very few spores are found which have produced a germ tube. Even after five days there is rarely any growth visible to the naked eye, but a microscopic examination shows a limited germination. Possibly 1 to 5 per cent of the spores are seen to have formed short germ tubes, very rarely branching. Very frequently the germinating spores showed peculiar swellings or knobs (appressoria?) at the end of the germ tube (fig. 1, A).

Several experiments were made by adding varying quantities of ether to sterile distilled water containing the spores, and very small amounts stimulated germination to a marked degree.

The germination of the sclerotia-like bodies, or sterile pycnidia, was also tested. With sterile forceps the upper portion of these erect aerial bodies was broken off and placed upon potato cylinders, corn-meal agar slants, etc. Growth occurred in practically all of the tests made with these bodies. Plates were also poured from corn-meal agar tubes, in which some of these sclerotial bodies had been placed, so that growth could be watched under the microscope. Plate 17, C, shows the appearance of a cross section of such a body after growth had started.

PHYSICAL AND BIOCHEMICAL CHARACTERS

REDUCTION OF NITRATES.—Cultures in Dunham's solution with potassium nitrate were tested after 5, 10, 15, and 20 days' growth by the starch-iodin method. No blue color reaction resulted, showing the absence of nitrites, as is also shown below under "Indol production."

INDOL PRODUCTION.—Tests for indol were made with cultures of varying age in Dunham's solution by means of the nitroso-indol reaction. No indol was produced at any time.

AMMONIA PRODUCTION.—Qualitative tests for ammonia were made with cultures grown in litmus milk, nutrient bouillon, and steamed corn meal by means of the calcium-hydroxid method. The results were positive in all cases, both in old and in young cultures.

VITALITY ON CULTURE MEDIA.—The fungus can live for a considerable period in flasks of corn-meal agar or on potato cylinders, but it loses its virulence rather quickly. Transfers on January 13, 1916, from corn-meal flasks made on January 29, 1915, grew promptly; but inoculations from these fresh transfers gave but a very small percentage of infections. In another experiment, 1 flask out of a series of 10 almost 2 years old sent out from its dried pycnidial or stromatic surface aerial hyphæ in about 10 days after the addition of freshly prepared sterile milk It was not possible to determine from what points these hyphæ were derived—that is, whether from the stroma or from the pycnidia or spores—since the growth began below the surface of the milk and was rather far advanced before it was observed. It is rather certain, however, that it was not from the old dried cirri, since 12 corn-meal flasks were inoculated from some of the dried extruded spores in this flask, previous to the addition of the milk, all with negative results.

TEMPERATURE RELATIONS.—The optimum temperature for growth lies between 20° and 25° C. Numerous tests were made on various media in all the compartments of a large thermostat. Germination will occur at much higher and lower temperatures, but the production of pycnidia and spores is in such cases very limited or absent. To test the effect of temperature on growth, spores were germinated on steamed-potato cylinders, corn-meal agar, etc., and then placed in the incubators at different temperatures, the range being from 1° to 37.5° C., where they were allowed to remain from two to five weeks. No growth occurred below 5°. No pycnidia were formed below 12°, and none above 28°. At 36° and 37.5° there was possibly a 10 per cent germination, but it was limited to the germ tube in most cases. The percentage of germination does not vary to any great extent between 18° and 35°, though the optimum point apparently lies very close to 25°.

MORPHOLOGY AND TAXONOMY

The hyphæ of the mountain laurel fungus are exceedingly fine, and it is located with difficulty in the tissues of its host. Nevertheless, this may be best accomplished by means of horizontal sections stained by Van Gieson's method.

The mycelium is septate, very fine, frequently branching, and hyalin. In the host the ends of the hyphæ are at times much swollen.

The pycnidia which form on leaves or stems of *K. latifolia* (Pl. 15, A, B) are very small compared to those on steamed white corn meal, and on steamed-potato cylinders. Those occurring on the leaves vary all the way from 75 to 500 μ in diameter. The majority, however, are small, usually about 250 μ in diameter. On steamed corn meal the pycnidia are from 0.5 to 5 mm. in diameter, the average being 2 mm. On the leaf of *K. latifolia* the color of the fruiting bodies is dark brown, almost identical with the color of the dead leaf tissue surrounding them. On culture media the color varies as stated elsewhere, being usually gray white or dark greenish olive to dark ivy-green. On the host the pycnidia are not deeply embedded, but are subepidermal and usually somewhat flattened and roundish (Pl. 15, A). There is no beak. No stroma is formed on leaves or stems. The outer wall of the pycnidium is rough and on sectioning is seen to be somewhat carbonaceous, of parenchymatous texture, and irregularly thickened, the thickest portion being toward the top (Pl. 15, D). The majority have but a single chamber.

The pycnidia on culture media are much larger than on the host. They are also much more irregular in form, but usually are roundish and somewhat flattened (Pl. 16). The carbonaceous wall is very much more pronounced than on the host. The outer surface is smooth and velvety in appearance. A cross-section of a pycnidium produced on steamed corn meal is shown in Plate 16, D, E. It is seen to be made up of very small pseudoparenchymatic cells, the majority of these cells being not over 10 μ in diameter. Usually the pycnidia on culture media are many-chambered. Frequently the chambering is limited to the outer portions of the pycnidium, and the cells are then coarser and show a more or less radial arrangement. Upon approaching the portion which is producing the spores, the parenchymatous structure is gradually lost, and only interlacing hyphæ appear, which are here very prominently nucleated (Pl. 16, E). The sporophores are borne upon a more or less carbonaceous mat of hyphæ.

STERILE PYCNIDIA.—In the cultural tests conducted, it has been found that a large number of the bodies formed (usually upon a stroma) are sterile. The structure of these bodies is shown in Plate 17, A, B. It is identical with the internal central structure of a pycnidium which is producing spores only at its margins. In all the numerous sections made of these sterile bodies, small areas have been found at different points along the periphery, showing interlacing hyphæ prominently nucleated (Pl. 17, B), and which suggested to the writer that these bodies were really potential pycnidia, since areas similar to this are the spore-producing portions of the pycnidia. Not much time has been expended in determining this point, but the following experiment was repeated several times, and in each test spores were produced in 75 to 100 per cent of the bodies used in the experiments. From 6 to 25 (in one experiment 60) of these sterile structures were selected from the

stromatic surface of a culture on steamed corn meal. With sterile forceps these were freed from the stroma, placed in a tray, and bisected. One-half of each of these pycnidia-like bodies was at once fixed in Carnoy's fluid, and later embedded in paraffin. The corresponding half in each case was placed in a petri dish of corn-meal agar, or of corn meal. They were examined daily, and in all the experiments more than two-thirds, and in four experiments all of the bodies transferred produced spores in from 4 to 10 days. Rapid growth of mycelium always occurred in the transfers to dishes of steamed corn meal, but only about 5 per cent produced spores. In this medium the pycnidial bodies become covered with a white, dense mat of hyphæ and the surrounding medium is likewise overgrown with a thick stromatic layer. Such is not the case when corn-meal agar is used; only a very delicate fringe-like growth around the pycnidial bodies occurs, and their surfaces are not overgrown. This again goes to show that the character of the medium has much to do with the form and the fertility of these structures. As soon as spores were extruded, the bodies were fixed in Carnoy's fluid, embedded in paraffin, and sectioned to determine from what portion the spores had been produced. It was found that the portions of the sterile bodies showing the nucleated hyphæ were the points from which the spores originated (Pl. 17, A). It has been observed in the cultural studies made that these sterile bodies are produced only when the culture medium is of such character as to stimulate extremely rapid and abundant vegetative growth—that is, the stromatic surface becomes crowded, and possibly the oxygen or moisture requirements are inadequate. It might also be a question of food, since between the actively growing spore-potential cells lie a mass of dead cells resting upon a hard, dry stroma, frequently raised above the surface of the medium.

The sterility of these bodies might, too, be due to the production of toxic substances by the rapid mycelial growth. The central portion of the flasks where growth is more rapid is usually where the majority of the sterile bodies occur, the more scattered pycnidia on the margins producing spores in an almost constant ratio.

It should be noted that the object in sectioning one half of these sterile bodies when placing the other half in the culture medium was to determine absolutely that they were not then producing spores, and also to study the structure of each for comparison with the other half after it had fruited.

A few experiments were made to determine the effect of limited air supply upon the production of spores by these sterile pycnidia. About 100 of these bodies were bisected and arranged in rows on petri dishes of corn-meal agar. One half of each sterile pycnidium was left uncovered in the dish and the other half covered with sterilized slides or cover glasses. After five to seven days all of the bodies left uncovered in the dish were extruding masses of spores (Pl. 16, C), while those covered had produced no spores and only about one-third as much mycelium.

Two kinds of spores are found both in the pycnidia on the host and in culture media: An egg-shaped to spindle-shaped spore, and a much longer, slender, curved, sickle-shaped, or hooked spore (fig. 1, B). The former are designated by Diedicke (*3*) as α spores, and measure unstained 5.5 to 8.8 by 1.8 to 3.6 μ, the average size being 2 by 5.7 μ. Stained, 5 to 5.8 by 1.1 to 1.2 μ. They are hyalin, nonseptate, contain two to three oil drops (tested with Sudan III), and a deeply staining nucleus. They are borne on straight or slightly curved basidia (fig. 2, B) measuring 0.5 to 2 by 9 to 20 μ. The second form of spore which is found in the same pycnidium with the α form, Diedicke calls β spores and Shear (*5*) scolecospores. Reddick (*2*) regards them as free paraphyses. Shear states that—

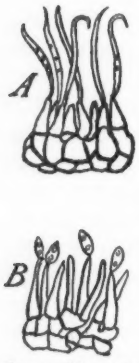

FIG. 2.—*Phomopsis kalmiae*: *A*, Scolecospores and basidia; *B*, the ordinary pycnospore and basidia. × 850.

> The term "scolecospore" is applied to these long, slender, crooked bodies tentatively to distinguish them from the ordinary pycnospores, because we regard them as reproductive organs, though they have not yet been found to germinate under the ordinary cultural conditions in which the other pycnospores germinate . . . true paraphyses are not normally abstricted and set free as these are, and occur typically only in connection with the asci, though the apparently sterile hyphae sometimes found intermixed with sporophores in pycnidia and acervuli, have been so designated by some mycologists. These slender, curved spores are evidently identical in character and origin with the so-called stylospores of Diaporthe as described by Nitschke [*1*], the other pycnospores being called spermatia by him.

The scolecospores are hyalin and contain one to several oil drops, often none, and most rarely a very pale-staining nucelus may be seen. They measure unstained 1.6 to 2.4 by 14 to 33.6 μ, average, 1.9 by 22 μ; stained, 0.6 to 0.8 by 13.2 to 23.1 μ. They are borne on short, tapering, occasionally almost pyriform basidia measuring 2.2 to 2.7 by 5.5 to 11.1 μ (fig. 2, A). The scolecospores occur in less number always than the other kind of spore and pycnidia have been found containing no scolecospores at all. No pycnidium has been found with only the scolecospores present, though there are chambers within the multichambered pycnidia which do contain only the scolecospores.

In some culture media a raised stroma is formed in which the pycnidia develop and in others the stroma is absent.

The spores are extruded in irregular droplike masses, cream, waxy-yellow or amber in color, or in definite threadlike form, measuring 10 to 25 mm. in length.

TECHNICAL DESCRIPTION OF THE FUNGUS

All attempts toward finding an ascogenous form of this fungus have been unsuccessful. Inasmuch as it differs from all other fungi so far described as infectious to *K. latifolia*, and as the genus in which it must

undoubtedly be placed from the characters given is Phomopsis, it is believed that the name "*Phomopsis kalmiae*" is a suitable one.

Phomopsis kalmiae, n. sp.

Pycnidiis in foliis et caulibus kalmiae latifoliae subglobosis, epidermide tectis, sparsis, sine stromate, fuscis, carbonaceis, ostiolatis, pleurumque unilocularibus. Pycnidiis in culturis subglobosis, vel saepe late ellipsoideis, non definite ostiolatis, plurilocularibus, sparsis, gregariis, vel in stromate aliquid denso, atro-viridibus, carbonaceis.

Sporulis ovatis, ellipsoideis, raro subfusoideis, continuis, hyalinis, typice 2-guttulatis, 1.8–3.6 × 5.5–8.8 μ. Basidiis filiformibus, pleurumque continuis, hyalinis, obtusis, 0.5–2 × 9–20 μ. Scolecosporulis filiformibus, attenuatis, fusoideis, rectis, vel leviter curvulis, vel hamatis, raro sigmoideis, saepe guttulatis, 1.6–2.4 × 14–33.6 μ. Basidiis brevibus, hyalinis, continuis, subulatis, 2.2–2.7 × 5.5–11.1 μ.

Pycnidia in leaves and stems scattered, subepidermal, brown, carbonaceous, usually unilocular, subglobose, without stroma, ostiolate. Pycnidia in culture media scattered, aggregate or in a rather thick stroma, subglobose to broadly elliptical, plurilocular, without definite ostiole.

Spores oval, ellipsoidal or rarely subfusoid, with two to several oil drops, continuous, hyalin, measuring 5.5 to 8.8 by 1.8 to 3.6 μ. Basidia filiform, tapering to blunt points, continuous, hyalin. Scolecospores filiform, attenuate, spindle-shaped and straight or slightly curved, or hooked and attenuate, occasionally delicately S-shaped, hyalin, continuous, and often contain one to several oil drops, measuring 1.6 to 2.4 by 14 to 33.6 μ. Basidia short, hyalin, continuous, subulate, 2.2 to 2.7 by 5.5 to 11.1 μ.

SUMMARY

The leafblight of *Kalmia latifolia* is characterized by a blight or dryrot involving large areas of either the leaf blade or the entire leaf. Later, it extends through the petioles into the stems and may eventually kill the entire plant. A fungus has been isolated whose parasitism has been demonstrated by successful inoculations into healthy plants. No published information upon this disease has been found, and the casual fungus is therefore described as a new species: *Phomopsis kalmiae*.

Pycnidia are readily produced on diseased leaves placed in damp chambers. Sclerotia-like bodies and pycnidia are produced in large numbers in most of the ordinary culture media. The sterile bodies are undoubtedly potential pycnidia as shown by the production of pycnospores after transplanting portions to fresh culture media.

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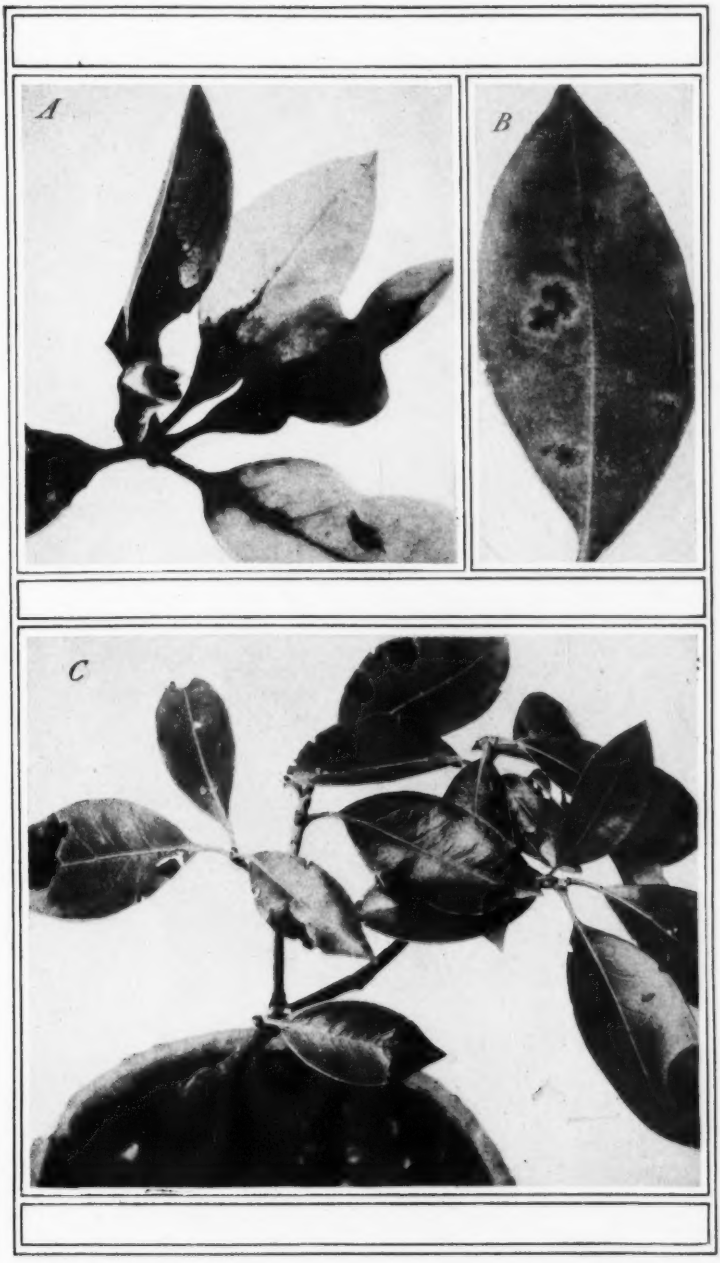
PLATE 14

A.—Twig of *Kalmia latifolia* showing late stage of infection with *Phomopsis kalmiae*. Inoculation of March 4, 1915. Slightly reduced.

B.—A leaf of *K. latifolia* in an incipient stage of infection. Note the lighter green area surrounding the diseased portions. Inoculation of January 6, 1916. About natural size.

C.—Plant of *K. latifolia* showing the intermediate stage of the disease. Reduced one-half. Photographed by Mr. James F. Brewer.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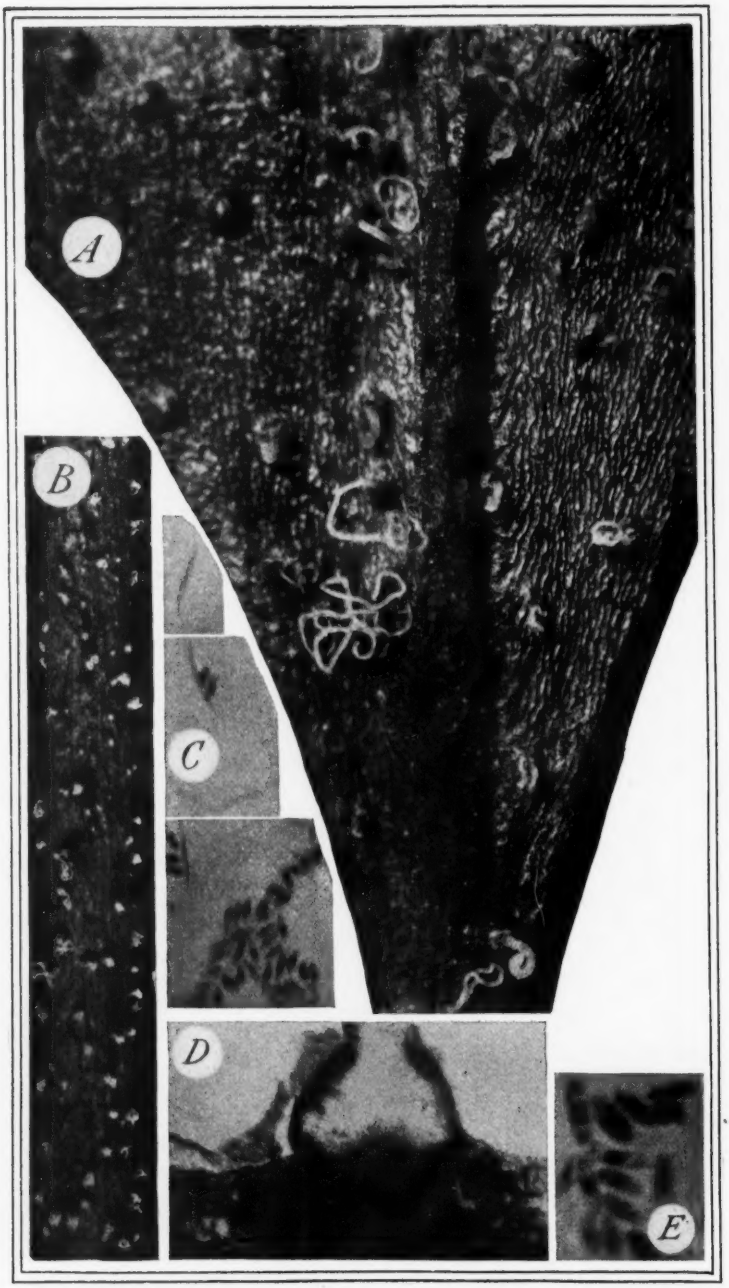
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PLATE 15

A.—A leaf of *Kalmia latifolia* enlarged 13 times to show the character of the pycnidia of the fungus on its host. Photographed by Mr. James F. Brewer.

B.—A stem of *Kalmia latifolia* 40 days after inoculation in leaves only. The plant was kept under very moist conditions, which favored the production of pycnidia. Spores in the form of cirri may be seen extruding from the pycnidia. ×5.

C.—Photomicrograph showing both kinds of spores of *Phomopsis kalmiae*, from culture on corn meal. Note the relative staining properties of the two types. A 12 ocular and a 4 objective were used. The stain was Delafield's hematoxylin and eosin. × 695. Photomicrograph by the writer.

D.—Section through a pycnidium of *Phomopsis kalmiae* on leaf of *K. latifolia*. Note the superficial character of the pycnidium and its ostiole, from which the spores lying to left have been extruded. × 92.

E.—The ordinary type of spore of *Phomopsis kalmiae* more highly magnified (12 ocular and 2 mm. oil-immersion lenses). Photomicrograph by the writer. × 1,200.

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PLATE 16

Phomopsis kalmiae:

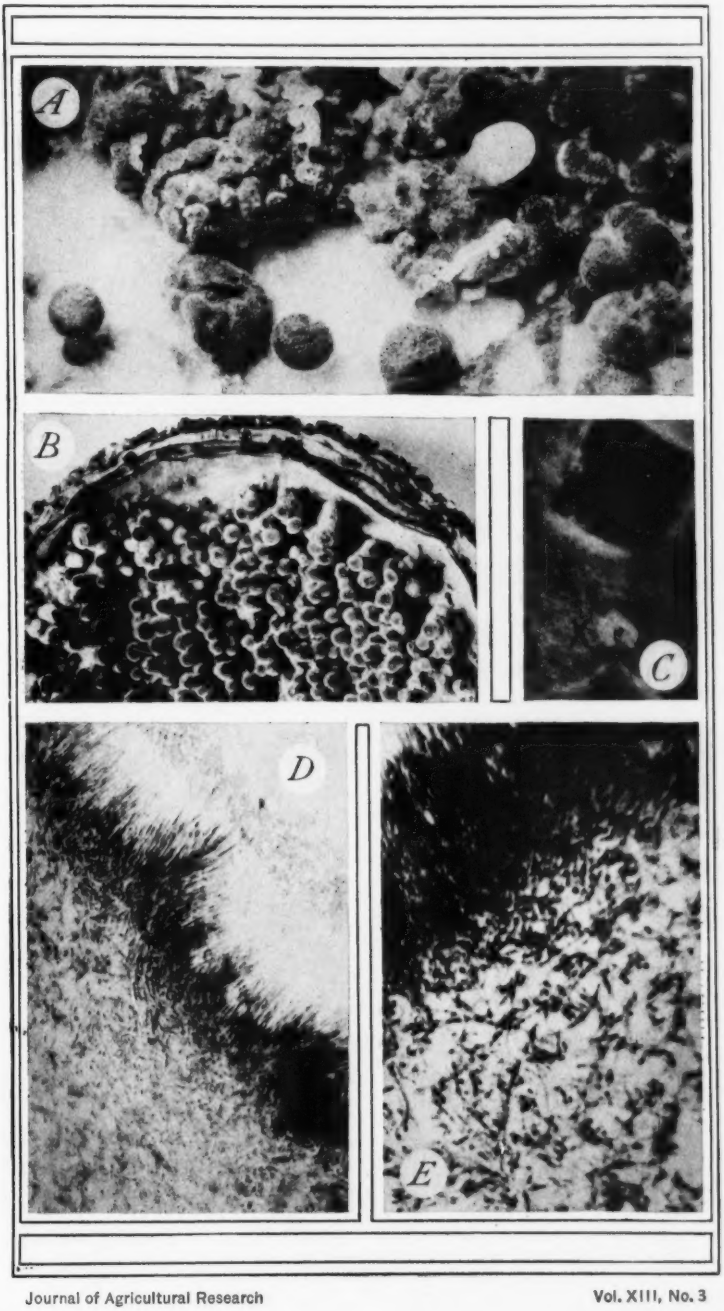
A.—An 18-day-old culture on steamed corn meal enlarged about 5 times. Note the extruding spore masses. Photographed by Mr. James F. Brewer. × 6.

B.—A 15-day-old culture on same medium, natural size. Later, the peripheral pycnidia extruded cirri freely, but the central ones did not. Photographed by Mr. James F. Brewer. × 1.1.

C.—Portion of corn-meal agar plate on which were sown three bisected sterile pycnidia. One half of each pycnidium was covered with sterile glass, and no spores were produced. The section left uncovered produced spores within five days, as here shown at X. Slightly reduced.

D.—Section through a pycnidium, showing the sporophores and the nucleated hyphæ just below them. × 175. Photomicrograph by the writer.

E.—A portion of same section shown in figure A more highly magnified; about 500. Photomicrograph by the writer.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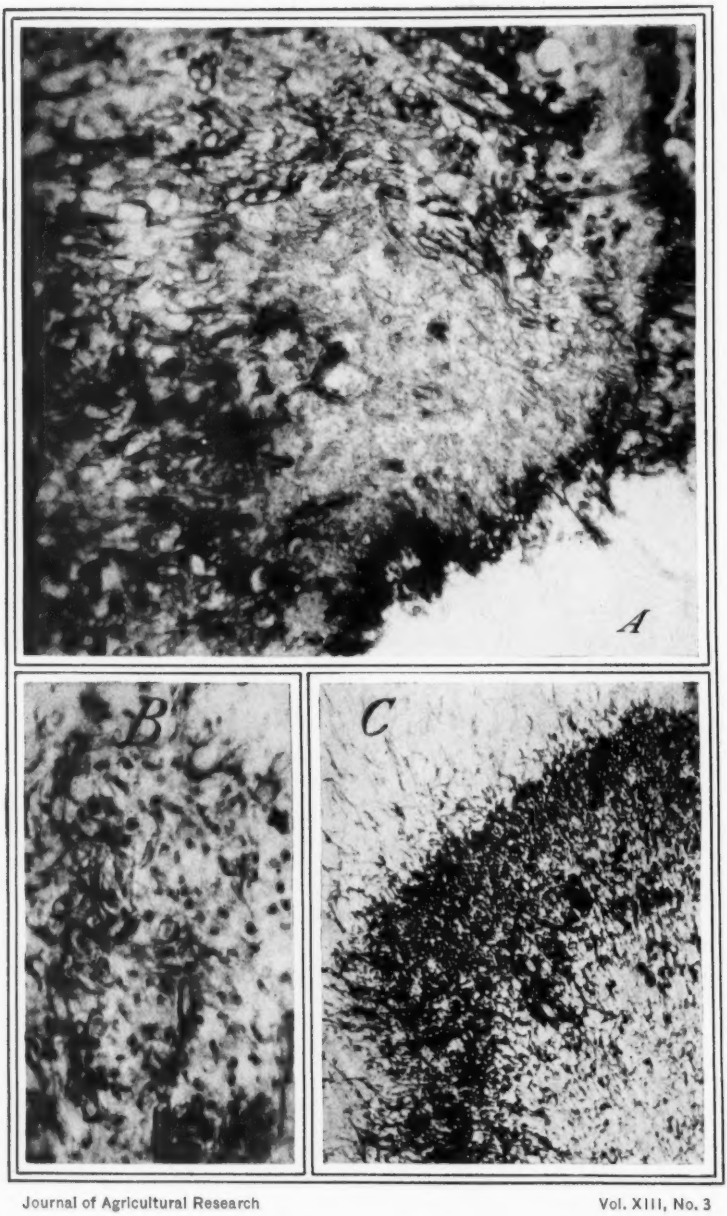
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PLATE 17

Phomopsis kalmiae:

A.—Section through a sterile pycnidium, showing an area containing nucleated hyphæ. From such areas spores arise when the pycnidium is transferred to a suitable medium. × 200.

B.—Central portion of figure 1 more highly magnified. × 625.

C.—Section through a sterile pycnidium, showing growth beginning at the margins after it had been transferred to a more suitable medium. × 110.

Photomicrographs by the writer.